ACTA PHYSIOLOGICA SCANDINAVICA
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FROM THE INSTITUTE OF NEUROPHYSIOLOGY,
MICHAELSEN INSTITUTE
UNIVERSITY OF COPENHAGEN, DENMARK

LOCAL ANESTHETICS

AN ELECTROPHYSIOLOGICAL INVESTIGATION OF LOCAL
ESIA OF PERIPHERAL NERVES, WITH SPECIAL
REFERENCE TO XYLOCAINE

by

JØRGEN RUD

With appendices on

DIFFUSION WITH SIMULTANEOUS INACTIVATION

and

ESTITUTION OF THE NERVE AFTER ANESTHESIA IN TERMS
OF DIFFUSION THEORY

by

P ROSENFALCK, M. SC

TRANSLATED

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M LENNOX, M D

COPENHAGEN 1961

Forsvaret finder sted i Medicinsk anatomisk
Institut's store auditorium, Nørre Allé 63,
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The investigation was carried out in the Institute of Neurophysiology, University of Copenhagen during the years 1949 to 1958

To the director of the institute, Professor FRITZ BUCHTHAL I should like to express my sincere thanks for giving me the opportunity of doing research, for providing excellent facilities and for constant encouragement and support

I am indebted to Mr POUL ROSENFALCK M Sc, the author of Appendix 2 and 3 of this paper, for introducing me to diffusion theory and for numerous and most helpful discussions My thanks are due to Docent OVE STEN KNUDSEN M D for much good advice and constructive criticism in the evaluation of the results and to Docent J C SKOU M D for valuable advice and discussion I also wish to thank Mr CHRISTIAN GULD E E who designed the electronic equipment used in this study and patiently guided me as to its application

Professor PALLE ANDERSEN kindly reviewed the sections concerning the Ringer s and anesthetic solutions and Mr OLE SVENSMARK M Sc taught me many details of physicochemical technique, Professor K G WINGSTRAND performed and evaluated the histological part of the fiber distribution and degeneration experiments and Docent ERNA CHRISTENSEN M D made histological sections of a nerve Mr VAGN ANDERSEN constructed the experimental chamber Mr FLEMMING RIIS has prepared most of the figures To them I offer my sincere thanks

The work has been supported by grants from Fonden til støtte for videnskabelige og praktiske undersøgelser inden for tandlægekunsten , 'Rektor, professor A Budtz Jørgensen og Hustru s studielegat til fremme af den odontologiske videnskab and The Danish State Research Foundation"

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Denne afhandling er af Københavns Tandlægehøjskole antaget til offentlig at forsvares for den odontologiske doktorgrad.

København, den 21. juni 1960.

P. O. Pedersen
Rektor

C. HAMBURGERS BOGTRYKKERI A/S
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CONTENTS

LIST OF SYMBOLS

B	xylocaine or procaine base
BH^+	xylocaine or procaine acid
C	concentration
C_B	concentration of xylocaine or procaine base
C_e	concentration of the active anesthetic outside the nerve
C_m	minimum concentration for xylocaine or procaine base
C_0	hydrochloride concentration of xylocaine or procaine
c p s	cycles per second
D	diffusion coefficient or velocity factor
K_a	thermodynamic ionization constant
K_s	acid constant
L_I	preanesthetic recording electrodes
l_I	preanesthetic recording electrodes distal electrode
1_I	preanesthetic recording electrodes proximal electrode
L_{II}	postanesthetic recording electrodes
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M	molar
Megohm	10^6 Ohm
m-equiv	milliequivalents
msec	10^{-3} second
m sec	meter per second
pK_a	negative logarithm of the thermodynamic ionization constant
r m s	root mean square
r_0	nerve radius
$T_{0.1}$	time from the moment when the nerve is just blocked and the anesthetic is substituted with Ringer's until the amplitude is 1 per cent of the initial value
$T_{0.5}$	time from the moment when the nerve is just blocked and the anesthetic is substituted with Ringer's until the amplitude has reached 50 per cent of the initial value

INTRODUCTION

Local anesthetics may be defined as agents which, when applied locally in concentrations without local or generalized toxic effects, reversibly arrest impulse conduction along the nerve fibers. Anesthetics affect nerve cells in the peripheral and central nervous system, muscles and neuromuscular transmission as well as peripheral nerves.

An essential aspect of the mode of action of local anesthetics is that they do not abolish the potential difference which exists across the membrane of a resting nerve fiber (HOBER et al. 1939, TASAKI 1953). Local anesthetics even cause a slight hyperpolarization of the membrane (BISHOP 1932, BENNETT and CHINBURG 1946, SHANES 1951a and STRAUB 1956a). This is in contrast to other anesthetics, such as ether for example, which block the nerve by depolarizing the membrane (LORENTE DE NO 1947). The local response as well as the propagated action potential disappear after application of local anesthetics (ROSENBLUETH and RAMOS, 1951, CASTILLO and STARK 1952 and BENNETT et al. 1942). Local anesthetics do not affect the uptake of oxygen in nerves (LARRABEE et al. 1947, BRINK 1951 as opposed to SHERIF 1930). Cocaine diminishes potassium uptake of normal nerves and nerves with inactivated "active transport" of ions to the same degree. The inactivation of active transport was obtained by anoxia and by monoiodo-acetic acid poisoning (SHANES and BERMAN 1956). This indicated that local anesthetics affect the passive transport of ions and not the active removal of sodium from the cell by the sodium pump. These findings as well as the absence of a depolarizing action of local anesthetics have led to the assumption that their blocking action is due to the fact that they prevent the increase in sodium conductance which normally is associated with the activitation of the nerve fiber (WEIDMANN 1955, STRAUB 1956b).

In myelinated nerve fibers local anesthetics act solely on the nodes of Ranvier, the myelin sheath preventing contact with the nerve membrane (KATO 1936, TASAKI 1939 and WOLFGRAM and HARREVELD 1952). There is evidence of a synergism between calcium ions and local anesthetics (FLECKENSTEIN and HARDT 1949). Thus procaine can repolarize a membrane

$t_{0.5}$ time from the application of the anesthetic until the amplitude has been reduced to half

$t_{0.01}$ time from the application of the anesthetic until the amplitude has been reduced to 1 per cent of its initial value

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nerve during the course of local anesthesia provides a direct measure of the progress of anesthesia This method was employed by Bennett et al (1912) who measured the time to 80 per cent reduction in action potential amplitude

An investigation of the time course of anesthesia is of interest for the following reasons

1) The effectiveness of a local anesthetic is determined by its minimum concentration and by the rapidity with which it penetrates the nerve A simple and sufficiently precise method for determining these two parameters would be valuable Ehrenberg (1948) attempted to develop such a method He applied various concentrations of the anesthetic to the nerve of a nerve-muscle preparation and measured the blocking time It is in principle possible to determine both the minimum concentration and the diffusion coefficient on the basis of the assumption that the substance penetrates the nerve by free diffusion Ehrenberg (1948) disregarded a number of complicating factors in the experimental conditions in question However his method does allow an extremely simple determination of two of the quantities which characterize local anesthetics

An analysis of the extent to which the course of anesthesia may be described in terms of free diffusion is of interest since a satisfactory agreement would allow prediction of the time course in a whole nerve with various concentrations of a local anesthetic knowing the minimum concentration and the diffusion coefficient It seemed therefore of interest to investigate in greater detail the time course of anesthesia and of restitution after anesthesia for various outer concentrations of the anesthetic to obtain information as to how closely the events may be described in terms of a simple diffusion process Such a study of the time course during the whole of anesthesia has been undertaken in the study reported here

2) Recording the action potential amplitude during anesthesia of nerves some with and some without sheath an attempt was made to determine the effect of the nerve sheath as diffusion barrier

3) In analyzing the time course of anesthesia in a nerve it must be known which portion of the molecule of the anesthetic is the active anesthetic component Previous investigations of this problem (Gros 1910 Trevan and Boock 1927 Gardner Semb and Graham 1931 and Ehrenberg 1948) are unanimous that it is the uncharged base component of the local anesthetic molecule which is active as anesthetic agent

However Skou (1951) has found from Trevan and Boock s (1927) and his own investigations that the minimum concentration expressed as the concentration of base varies with pH which could indicate that the concentration of base is not the sole determinant of anesthetic effect In the in

which is depolarized by a calcium free solution (STRAUB 1956b) and cocaine can arrest the *spontaneous activity brought about by precipitation of* calcium (SHANES 1951a). That lack of calcium ions affects nerve conduction by increasing the permeability of the membrane to sodium (STAMPFLI and NISHIE 1956, HASHIMURA and WRIGHT 1958, MUELLER 1958) is of special interest, considering the assumed ability of local anesthetics to prevent the increase in sodium conductance which accompanies the normal nerve impulse and thus to block it. In contrast to the synergism between calcium ions and local anesthetics there are signs of an antagonism between the effect of local anesthetics and of veratrine (BUCHTHAL and LINDHARD 1942, SHANES 1950, 1951b, STRAUB 1951, 1956c). Thus local anesthetics can restore impulse conduction in a nerve blocked by veratrine (FLECKENSTEIN 1951). Veratrine increases the sodium permeability of the resting membrane (SHANES 1951b, STRAUB 1951) and the antagonism might be explained by the inhibitory action of local anesthetics on sodium permeability.

The investigations of KATO (1936), TASAKI and TAKEUCHI (1942) and TASAKI (1953) have shown that a local anesthetic applied to a single nerve fiber blocks impulse conduction instantaneously when the concentration of the anesthetic is above a certain concentration, the minimum concentration. A whole nerve is blocked by concentrations above the minimum concentration of a local anesthetic after a time interval which becomes shorter with increasing concentration of the anesthetic relative to the minimum concentration (EHRENBERG 1948). This is due in part to the fact that a certain time is required for the substance to diffuse into the nerve. To evaluate the suitability of a local anesthetic it is thus necessary to know its minimum concentration, the diffusion rate into the nerve and the toxic concentration. The usual clinical tests of local anesthetic effect, such as the corneal test and the subcutaneous or intradermal wheal test, do not determine these characteristics accurately because the experimental conditions cannot be held constant and because the anesthetic effect is reported subjectively. Most of the physiological investigations as to the effects of local anesthetics have therefore been undertaken with nerve muscle preparations, the absence of muscle concentration serving to indicate full anesthesia. This method is entirely satisfactory to determine the blocking time, i.e. the time when all motor nerve fibers to the muscle in question have ceased to conduct an impulse. The method does not allow the course of anesthesia in the nerve to be followed since its correlation with the degree of contraction is not known. Furthermore only the effect on motor fibers, comprising less than half the fibers in a mixed nerve, is investigated by this method.

Recording changes in the amplitude of the action potential of a whole

CHAPTER 1

METHOD

Preparation of the nerves.

The nerves used were sciatic nerves of Hungarian frogs, *Rana esculenta*. The nerve was isolated close to the spinal cord and up to a point about 18 mm distal to the division of the peroneal and tibial nerves. Branches were severed about 5 mm from the nerve.

During dissection the nerve was frequently moistened with a Ringer's solution of the same pH as the anesthetic solution to be used in the experiment. After dissection the nerve was immersed for an hour in this Ringer's solution.

Dissection of the nerve sheath.

A series of experiments was carried out on sheathless nerves. The nerve sheath was dissected away from more than 10 mm of the distal part of that portion of the nerve to be immersed in the anesthetic solution. That the sheath was removed in its entirety was demonstrated by ensuring that it was possible to draw single fibers out of the nerve. That the sheath was entirely removed was also checked by determining the interference colors of the nerve in a polarizing microscope with a red 1st order gipsum plate. Under these conditions the nerve sheath appeared blue with the slow ray of the compensator lying along the fiber axis while the nerve fibers appeared yellow.

Comparing the nerve diameter before and after dissection of the sheath, the thickness of the sheath was found to be 0.01-0.03 mm in agreement with the findings of LORENTE DE NÓ (1950).

Experimental chamber.

The excised nerve was placed in an experimental chamber, where a portion of it could be held submerged in the test solution by means of a perspex pin similar to the procedure used by LARRABEE and POSTERNAK (1952) (see Fig. 1).

2

vestigation presented here an attempt was made to contribute to this problem by a) investigating the minimum concentration of xylocaine and procaine at various pH levels and b) investigating the effect of varying the pH on the time course of anesthesia and of restitution the concentration of base being constant and c) investigating the time course of anesthesia and restitution at various concentrations of base with the same hydrochloride concentration at different pH

4) The recording of the action potential offers the advantage that one can investigate the effect of the anesthetic on the nerve in subminimal concentrations In the present study the effect of subminimal concentrations on conduction velocity was determined

5) In these investigations xylocaine (lidocaine leostesin lignocaine) was used one of the most commonly employed anesthetic substances since Lofgren first description of it in 1946 For comparison a smaller number of experiments was carried out with procaine (novocaine) The results are discussed in relation to the clinical use of local anesthetics

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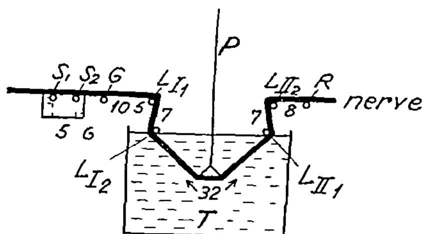


Fig. 1. Experimental chamber and placement of electrodes. N: nerve; S_1 and S_2: stimulating electrodes; G: ground electrode; L_I and L_{I_2}: pre-anesthetic recording electrodes; L_{II} and L_{II_2}: post-anesthetic recording electrodes. P is a perspex rod which holds the nerve submerged in the test vessel T containing the anesthetic. Electrode R is used in the resistance measurements.

The numbers on the diagram indicate the distances in mm. The nerve is stimulated at its peripheral end.

In this way it was possible to subject a well defined portion of the nerve to the anesthetic without risking spread of the test solution along the nerve. The nerve was stimulated at its peripheral end (see page 36) and the resulting action potential recorded just before the nerve entered the test vessel and just after it emerged. Thereby it was possible to follow the effect of the anesthetic both on the amplitude of the action potential and on conduction velocity in the affected section of the nerve lying between the two pairs of recording electrodes. Further details of the experimental chamber are described in Chapter 3 (p. 57).

The experimental chamber was constructed of perspex and designed as a moist chamber. The electrodes consisted of 0.5 mm platinum wires whose bared tips were 3 mm long, except at the tips they were embedded in perspex coated with silicone. Insulation from the moist walls of the chamber was necessary to avoid artefacts. The placement of the electrodes is shown in Figure 1.

To mount the nerve the front wall of the chamber was removed and the test vessel was lowered. The slightly stretched nerve was secured at each end by a cotton thread attached to hooks on the side walls of the chamber. The test vessel was filled with 10 ml of fluid from a syringe and a rubber tube through an opening in its bottom, the fluid surface just touching the lower electrode of each recording electrode pair, L_{I_2} and L_{II}. Between electrode L_I and L_{II} the nerve was held down in the test vessel by means of a perspex pin (*P*). The inner walls of the test vessel were treated with silicone to allow complete removal of all fluid. The walls of the test vessel were double to allow regulation of the temperature by circulating water at the desired temperature. To record the temperature and the pH in the test fluid a thermistor, a glass electrode and a reference electrode could be introduced through the top of the chamber.

Amplifiers

The action potentials were amplified with an A.C. amplifier with differential input. The amplifier had an input impedance of 100 $M\Omega$ in parallel with an effective capacity to ground of about $5 \mu\mu F$. The amplifier had adjustable maximum and minimum limiting frequency. It was found that distortion of the potential was insignificant when a lower limiting frequency of 1 c.p.s. and an upper limiting frequency of 10^7 c.p.s. were used. The noise level with such a frequency range was less than $2 \mu V$ r.m.s. and the common mode rejection of the amplifier was more than 10^4. The resulting common mode rejection of the amplifier plus electrodes is given on page 21.

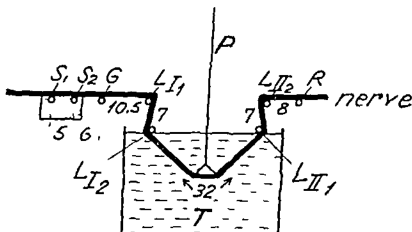


Fig 1 Experimental chamber and placement of electrodes N nerve S_1 and S_2 stimulating electrodes *G* ground electrode L_{I_1} and L_{I_2} pre anesthetic recording electrodes L_{II_1} and L_{II_2} post anesthetic recording electrodes *P* is a perspex rod which holds the nerve submerged in the test vessel *T* containing the anesthetic Electrode *R* is used in the resistance measurements

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when maximal amplification was used as at the postanesthetic recording electrodes the following experiments were performed to determine whether the degree of common mode rejection was adequate

Both recording electrodes were placed on the nerve Between the ground and the recording electrodes a 10 mm length of the nerve was substituted by a wool thread wetted with Ringer s solution to interrupt the propagation With the stimulation intensity and duration usually employed the amplification could be increased to the noise level without recording either action potential or electrotonus indicating a sufficient degree of common mode rejection

Recording of the nerve action potential

The amplified action potentials from the two pairs of electrodes were displayed simultaneously on the two beams of a double beam oscilloscope (Du Mont type 279)

Before during and after an experiment a train of signals of known amplitude was supplied from a laboratory generator Since the deflection of the cathode ray beam was not linear in the periphery of the screen (maximal deviation 6 per cent for amplitude and 13 per cent for time) the potentials were displayed as near the centre of the screen as possible Furthermore the distance on the screen was compared with the distance between the calibration signals at the same screen position

The oscilloscope screen was photographed on 35 mm film with a Fairchild camera Single sweeps were photographed on stationary film to record slow changes Rapid changes in action potentials were recorded on continuously moving film the stimuli being delivered once in two seconds The sweep velocity was 2 7 mm/msec Though the film moved continuously the rate was so slow (about 7 mm/sec) that there was no significant distortion of the record The time elapsed since the start of the experiment was obtained from the number of recordings Application of the anesthetic was marked on the continuously moving film

The action potentials were enlarged 5 to 8 times for measurement

Measurement of nerve diameter

The rapidity of the action of local anesthetics on nerve depends on the diameter of the nerve Measurement of the nerve diameter was carried out through the transparent side walls of the closed chamber In this way drying of the nerve during measurement was prevented The nerve diameter was measured immediately proximal to electrode L_1 the thinnest portion of the nerve affected by the anesthetic where blocking first takes place

Stimulation.

The rectangular stimulating pulses of 0.15 *msec duration* were passed through a double screened transformer to isolate the stimulator from the stimulating electrodes The stimulating electrode closest to the recording electrode was cathode. The stimulus strength was supramaximal for all fibers, in other words two or three times the strength which evoked an action potential of maximum amplitude The stimulator output was 2–20 volts but the actual stimulating current cannot be given because of the fluid shunt between the stimulating electrodes The oscilloscope sweep was synchronized from the stimulator. Single pulses could be delivered automatically every other second to record the rapid changes in the action potential

Artefacts in recording of action potentials.

Since the stimulator and amplifiers were in electrical connection through the nerve, there was a possibility of recording a stimulus artefact superimposed upon and distorting the action potential In experiments where high amplification was used it was therefore of importance to diminish or eliminate the stimulus artefact This could be obtained by 1) isolating the stimulating electrodes from ground 2) grounding the nerve at a point between stimulation and recording and 3) making use of a high common mode rejection of the amplifier with electrodes

Concerning 1 The stimulating electrodes were isolated from ground by passing the stimulating current through a transformer To ensure effective isolation at high frequencies the primary and secondary screens of a General Radio transformer (type 578–A) were connected as described by BUCHTHAL, GULD and ROSENFALCK (1955) (Fig 2) with one screen connected to the nerve through electrode *C* and the other screen connected to ground

Concerning 2 The ground electrode *G* was placed on the nerve (Fig 1) at a distance of more than 6 mm from the nearest recording electrode Moving the electrode along the nerve it was found that with a given common mode rejection an artefact was recorded when the ground electrode was less than 6 mm from the recording electrode L_{1}. The closer the ground electrode was advanced to the recording electrode the greater was the artefact

Concerning 3 It was important to prevent recording of the potential across the ground electrode common to both recording electrodes This was ensured by increasing the common mode rejection of the amplifier and electrodes BUCHTHAL, GULD and ROSENFALCK (1951) have shown that nerve and electrode impedances have a marked effect on the degree of common mode rejection Since a sufficient common mode rejection was essential

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Concerning 3. It was important to prevent recording of the potential across the ground electrode common to both recording electrodes. This was ensured by increasing the common mode rejection of the amplifier and electrodes. BUCHTHAL, GULD and ROSENFALCK (1951) have shown that nerve and electrode impedances have a marked effect on the degree of common mode rejection. Since a sufficient common mode rejection was essential

From the potential over the nerve (E_N) and the potential (E_R) over the known resistance (R) the nerve resistance was found as $R_N = R \cdot E_N/E_R$

In checking the accuracy of the method with a known resistance in place of the nerve the mean error of 10 measurements was ± 0.75 per cent. Measuring the resistance of a nerve in air 10 times at two places the mean error was ± 3.8 and ± 4.3 per cent

Determination of the pH of the test fluid

Since the effect of local anesthetics varies with the hydrogen ion concentration of the solution it was necessary to measure the pH of the solution continuously during the experiments

pH was measured with a pH meter (Radiometer type P H M 22) The glass electrode (Radiometer G 222 A diameter 1.5 mm resistance 2–300 MΩ at 20°C) was introduced directly into the test fluid The calomel potassium chloride reference electrode was introduced into a beaker containing saturated potassium chloride and contact with the test fluid was obtained through a glass tube 10.5 cm long and with a 2 mm lumen filled with Ringer agar (99 per cent Ringer s solution and 1 per cent agar) Because of this liquid junction the measured pH was 0.25 units greater than if measured with the reference electrode directly in the test chamber The deviation was constant over the pH range 4–8

The pH apparatus including the Ringer agar junction was adjusted using potassium hydrogenphthalate 0.05 molar (pH 4.005) and sodium borate 0.05 molar (pH 9.18) as buffer solutions

Humidity and temperature in the experimental chamber

Humidity To avoid drying of the nerve the air in the chamber had to be saturated with moisture This was obtained by bubbling an oxygen carbon dioxide mixture through four water bottles using special glass filters which gave very small bubbles When the bottles were placed in a water bath at 10–30° C a supersaturated moisture was obtained at the temperature of the chamber The supersaturated air was conducted through insulated tubes to the chamber Furthermore a sponge wetted with Ringer s solution was placed on the floor of the chamber To ascertain that full saturation of the air in the chamber was maintained a thermo couple wrapped in moist cotton was introduced into the chamber At constant temperature in the chamber no evaporation from the cotton was seen over a period of many hours

The diameter was measured microscopically with 21 times magnification using an ocular micrometer with movable cobweb calibrated against an object micrometer (subdivided into 1/100 mm) The diameter was expressed as the mean of 10 measurements The mean error of 10 measurements was ± 0 17 per cent

Measurement of resistance.

The resistance between the recording electrodes affects the amplitude of the action potential (see p 41) To determine whether the resistance changed in the course of an experiment and to correct for any such change it was necessary to be able to measure the resistance Measurement with a bridge arrangement has the disadvantage that the electrode impedances are included, therefore the following procedure was used the nerve was placed in series with a known resistance A suitable impulse from a current generator was introduced through this circuit From the fall in potential between the recording electrodes on the nerve and from the fall in potential over the known resistance, the resistance between the recording electrodes could be calculated (Figure 2)

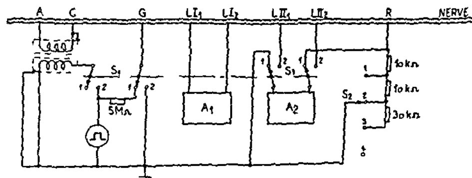


Fig 2 Arrangement of electrodes for stimulation recording of action potentials and measurement of resistance between recording electrodes

For the recording of action potentials switch S_1 is in position 2 and switch S_2 in position 1 The stimulating current passes through the double screened transformer to the nerve between the electrodes A (anode) and C (cathode) Electrode G is grounded and amplifiers A_1 and A_2 are connected with electrodes L_{I_1} and L_{I_2} for the pre anesthetic recording and with electrodes L_{II_1} and L_{II_2} for the post anesthetic recording

For the measurement of resistance S_1 is in position 1 and S_2 in position 1 2 or 3 With the stimulator as generator a current is passed via electrode G the nerve electrode R and a known resistance to ground Amplifier A_1 records the potential over the portion of nerve between electrodes L_{I} and L_{I_2} where it is desired to measure the resistance and amplifier A_2 records the potential over the known resistance

of the 10 nerves were totally blocked and the majority of the remaining nerves showed a substantial reduction in action potential amplitude

To ensure that the nerves which were blocked after anesthesia were undamaged, the nerves were transferred again to Ringer's solution Only those nerves which showed restitution of the action potential were included in the experimental material

Temperature in the test vessel was measured with a standard thermistor (Standard Electric, type U), which was placed as one resistance in an alternating current bridge. The tip of the thermistor was in contact with the nerve. The temperature could be read with an accuracy better than 0 1°C

Because of the high temperature of the oxygen-carbon dioxide which was passed through the chamber the temperature of the nerve had a tendency to rise during the experiment This rise in temperature could be compensated by running cool water through the double wall surrounding the test chamber Different temperatures were obtained by running water of a suitable temperature through the double wall of the experimental chamber In these experiments the liquid in the test vessel was stirred by means of a motor-driven propeller

Measurement of minimum concentration.

To avoid too long an experimental time, Hungarian frogs were used (Rana esculenta) small in size and therefore with thin nerves (about 0 30 mm in radius) Immediately after dissection the nerves were placed for one hour in Ringer's solution at the pH to be used during anesthesia Thereafter each nerve was mounted singly in the experimental chamber (Figure 1), lying from the site of the stimulating electrodes S_1 to S_2 across the ground electrode to the recording electrodes L_{II_2} and L_{II_1} In every series of experiments the action potential was recorded for each of 10 nerves in Ringer's solution whereafter the 10 nerves were mounted in such a way that the distal end of each nerve (the site of stimulation) was contained in a Ringer filled tube 20 mm long sealed at each end with vaseline The approximate 15 mm long proximal section of each nerve was suspended slightly stretched by cotton thread and was immersed in the anesthetic solution

The longest blocking time measured in 16 experiments with various xylocaine concentrations was two hours (experiment 156, C_B — 0 15 mM) It was therefore considered that an experimental time of six to eight hours should be sufficient to attain diffusion equilibrium between the bathing fluid and the interfibrillar fluid of the nerves (see p 76)

To maintain a constant pH the anesthetic solution was bubbled with oxygen carbon-dioxide mixture (by means of glass filters) during the whole anesthetic period

After 6–8 hours in the anesthetic solution the nerves were replaced in the experimental chamber and the degree of block in each nerve was determined from the amplitude of the action potentials evoked The minimum concentration was determined as the smallest concentration at which about half

of the 10 nerves were totally blocked and the majority of the remaining nerves showed a substantial reduction in action potential amplitude

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The usual sodium content of the Ringer s solution was therefore decreased from 115 mM to 75 mM The effect of this reduction in sodium concentration on nerve activity is discussed on p 50 Up to 40 mM of anesthetic could be added to this solution without altering its isotonicity The Ringer s solution as well as the anesthetic solutions with concentrations less than 40 mM had to be supplemented with an inactive component until the osmotic pressure corresponded to 249 m equiv Glucose might be considered as an inactive component LORENTE DE NÓ (1947) states however, that glucose can produce irreversible changes in nerves in the presence of anoxia Choline chloride was therefore used instead which according to LORENTE DE NÓ (1947) has no effect on the nerve impulse

Protolysis of xylocaine and procaine acid

The protolytic equilibrium (1) gives

$$\frac{a_B \quad a_{H\,O}}{a_{BH}} = \frac{C_B \quad f_B \quad a_{H\,O}}{C_{BH} \quad f_{BH}} = K_a \qquad (2)$$

where K_a is the thermo dynamic ionization constant a denotes the activity and C the molar concentrations of the substances involved in the equilibrium f_B and f_{BH} are the activity coefficients

Since B is uncharged f_B is assumed equal to 1 In addition

$$C_{BH} + C_B = C_0 \qquad (3)$$

C_0 representing the concentration of xylocaine or procaine hydrochloride (2) may therefore be written

$$C_B = \frac{C_0}{\frac{a_{H_3O}}{K_a \quad f_{BH}} + 1} \qquad (4)$$

From this expression the concentration of free base (C_B) may be derived when the following quantities are known

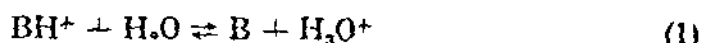
1) the concentration of xylocaine or procaine hydrochloride (C_0)
2) the hydrogen ion activity ($a_{H\,O}$)
3) the thermodynamic ionization constant (K_a)
4) the activity coefficient (f_{BH})

Ad 1 The anesthetic solutions were prepared with a known concentration of xylocaine or procaine hydrochloride

Chapter 2

ANESTHETIC AND RINGER'S SOLUTIONS

Local anesthetics are alkaloids which in the presence of acids form stable salts easily soluble in water. In this study only xylocaine and procaine hydrochloride were studied. In the concentrations used the salt was completely dissociated into acid and Cl^-. In watery solution the following protolytic equilibrium obtains



$$BH^+ + H_2O \rightleftharpoons B + H_3O^+ \qquad (1)$$

where B denotes the free base which is uncharged and BH^+ denotes the acid. More salt is formed when hydrogen ions are introduced into the solution while the concentration of the base is increased when the solution is rendered basic. Solutions with different concentrations of the free base could be obtained by changing the salt concentration or by varying the pH of the solution (see p. 29).

To maintain a constant concentration of free base during the experiments it was necessary to maintain a constant pH and buffer solutions were added to ensure a high buffer capacity. One of the physiological buffers is carbon dioxide-sodium bicarbonate which regulates the intracellular hydrogen concentration (AHLGREN 1930). In addition carbon dioxide is thought to have a specific effect on the metabolism of nerve (see p. 18). Three per cent carbon dioxide bicarbonate buffer was therefore added to the solutions (see p. 31) in addition to a phosphate buffer to obtain a sufficient buffer capacity (see p. 31).

To avoid the effect of other factors than the concentration of the anesthetic and the pH of the solution, all solutions, both Ringer's and anesthetic, were adjusted with respect to the osmotic pressure (249 m-equiv) and a constant concentration of Ca^{++}, K^+ and Na^+ while the concentration of Cl^- could be varied.

When the anesthetic was added to Ringer's solution it was necessary to reduce the total concentration of sodium chloride to maintain isotonicity.

The usual sodium content of the Ringer's solution was therefore decreased from 115 mM to 75 mM. The effect of this reduction in sodium concentration on nerve activity is discussed on p. 50. Up to 40 mM of anesthetic could be added to this solution without altering its isotonicity. The Ringer's solution as well as the anesthetic solutions with concentrations less than 40 mM had to be supplemented with an inactive component until the osmotic pressure corresponded to 219 m-equiv. Glucose might be considered as an inactive component. LORENTE DE NÓ (1947) states, however, that glucose can produce irreversible changes in nerves in the presence of anoxia. Choline chloride was therefore used instead, which according to LORENTE DE NÓ (1947) has no effect on the nerve impulse.

Protolysis of xylocaine and procaine acid.

The protolytic equilibrium (1) gives

$$\frac{a_B \cdot a_{H_3O^+}}{a_{BH^+}} = \frac{C_B \cdot f_B \cdot a_{H_3O^+}}{C_{BH^+} \cdot f_{BH^+}} = K_a \tag{2}$$

where K_a is the thermo-dynamic ionization constant, a denotes the activity and C the molar concentrations of the substances involved in the equilibrium. f_B and f_{BH^+} are the activity coefficients.

Since B is uncharged, f_B is assumed equal to 1. In addition

$$C_{BH^+} + C_B = C_0 \tag{3}$$

C_0 representing the concentration of xylocaine or procaine hydrochloride (2) may therefore be written

$$C_B = \frac{C_0}{\dfrac{a_{H_3O^+}}{K_a \cdot f_{BH^+}} + 1} \tag{4}$$

From this expression the concentration of free base (C_B) may be derived when the following quantities are known:

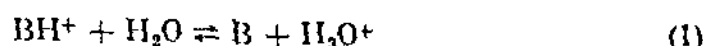
1) the concentration of xylocaine or procaine hydrochloride (C_0)
2) the hydrogen ion activity ($a_{H_3O^+}$)
3) the thermodynamic ionization constant (K_a)
4) the activity coefficient (f_{BH^+})

Ad 1. The anesthetic solutions were prepared with a known concentration of xylocaine or procaine hydrochloride.

CHAPTER 2

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$$BH^+ + H_2O \rightleftharpoons B + H_3O^+ \qquad (1)$$

where B denotes the free base which is uncharged and BH^+ denotes the acid More salt is formed when hydrogen ions are introduced into the solution, while the concentration of the base is increased when the solution is rendered basic Solutions with different concentrations of the free base could be obtained by changing the salt concentration or by varying the pH of the solution (see p 29)

To maintain a constant concentration of free base during the experiments it was necessary to maintain a constant pH and buffer solutions were added to ensure a high buffer capacity One of the physiological buffers is carbon dioxide-sodium bicarbonate which regulates the intracellular hydrogen concentration (AHLGREN 1930) In addition carbon dioxide is thought to have a specific effect on the metabolism of nerve (see p 18) Three per cent carbon dioxide-bicarbonate buffer was therefore added to the solutions (see p 31) in addition to a phosphate buffer to obtain a sufficient buffer capacity (see p. 31)

To avoid the effect of other factors than the concentration of the anesthetic and the pH of the solution, all solutions, both Ringer's and anesthetic were adjusted with respect to the osmotic pressure (219 m equiv) and a constant concentration of Ca^{++}, K^+ and Na^+, while the concentration of Cl^- could be varied

When the anesthetic was added to Ringer's solution, it was necessary to reduce the total concentration of sodium chloride to maintain isotonicity

phate buffer, the ionic concentration is increased. With the phosphate buffer



$$NaH_2PO_4 + NaOH = Na_2HPO_4 + H_2O \qquad (10)$$

with a concentration of NaH_2PO_4 of 0.0066 M, only NaH_2PO_4 is present at low pH (ionic strength of 0.0066). At high pH only Na_2HPO_4 is present with an ionic strength of 0.0198, in other words an *increase* in ionic strength of the Ringer's solution of 0.0132. At a mean value of pH (6.82) the increase in ionic strength from the phosphate buffer is 0.0066. This added to the ionic strength of 0.127 for Ringer's solution results in a total ionic strength of 0.134. Substituting this ionic strength in the DEBYE HÜCKEL equation ((7) p. 28) the activity coefficient $f_{BH^+} = 0.73$.

In the experiments C_0 values were used of 1, 2.5, 20 and 40 mM. The corresponding values for C_B at various pH levels may be calculated from equation (4) (Fig. 3).

Fig. 3. Protolysis of (*A*) xylocaine and (*B*) procaine acid at various pH values.
Ordinate: logarithm of the base concentration in mM.
Abscissa: pH.

The curves represent various initial *concentrations* of xylocaine and of procaine hydrochloride (C_0) in mM at 25°C.

Ad 2 The hydrogen ion activity may be determined by measuring the pH of the solution, since the method used for measuring pH gives the following relationship according to DOLE (1941)

$$pH = -\log a_{H_3O^+} \tag{5}$$

Ad 3 LÖFGREN (1948) found the thermodynamic ionization constant K_a for xylocaine acid to be $10^{-7.85}$ at 25°C K_a for procaine acid was calculated from the results of experiments performed by EISENBRAND and PICHER (1938) They measured the pH of various procaine solutions to which sodium hydroxide had been added Calculating the ionic strength (μ) for these solutions, and using the value $k_s = 10^{-8.95}$ found by EISENBRAND and PICHER (1938), it is found that $K_a = 10^{-8.93}$, using the formula of HÄGG (1948) which is based on the fact that pH measurements reflect the hydrogen ion activity

$$pk_s = pK_a + 0.5\,(z_s^2 - z_b^2)\,\sqrt{\mu} \tag{6}$$

with z_s and z_b as the valencies for the acid and the base and μ ionic strength

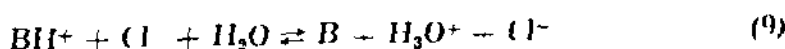
Ad 4 The activity coefficient (f_{BH^+}) is calculated with fair approximation, using the DEBYE-HÜCKEL equation

$$-\log f = \frac{0.5\,z^2\sqrt{\mu}}{1 + \sqrt{\mu}} \tag{7}$$

with μ representing the ionic strength of the whole solution
μ may be calculated from

$$\mu = 0.5\,\Sigma C_i z^2 \tag{8}$$

when C is the concentration of the single ions in solution and z their valency
Frog Ringer's solution contains the following salts NaCl 115 mM KCl 2.7 mM, $CaCl_2$ 1.8 mM and $NaHCO_3$ 3.7 mM *Substituting some of the* sodium chloride in the Ringer's solution with other salts containing 2 ions with valency 1, there is no change in ionic strength as long as the total concentration is the same This is the case for choline chloride, approximately so for sodium bicarbonate as well as for xylocaine and procaine hydrochloride From the dissociation of the salt



$$BH^+ + Cl^- + H_2O \rightleftarrows B + H_3O^+ + Cl^- \tag{9}$$

it is seen that it makes no differences how the equilibrium is shifted, there will only be two charged ions with one charge each In substituting phos-

phate buffer the ionic concentration is increased With the phosphate buffer



$$NaH_2PO_4 + NaOH = Na_2HPO_4 + H_2O \quad (10)$$

with a concentration of NaH_2PO_4 of 0 0066 M only NaH_2PO_4 is present at low pH (ionic strength of 0 0066) At high pH only Na_2HPO_4 is present with an ionic strength of 0 0198 in other words an *increase* in ionic strength of the Ringer s solution of 0 0132 At a mean value of pH (6 82) the increase in ionic strength from the phosphate buffer is 0 0066 This added to the ionic strength of 0 127 for Ringer s solution results in a total ionic strength of 0 134 Substituting this ionic strength in the DEBYE HÜCKEL equation ((7) p 28) the activity coefficient $f_{BH} = 0\ 73$

In the experiments C_0 values were used of 1 2 5 20 and 40 mM The corresponding values for C_B at various pH levels may be calculated from equation (4) (Fig 3)

Fig 3 Protolysis of (*A*) xylocaine and (*B*) procaine acid at various pH values.
Ordinate logarithm of the base concentration in mM
Abscissa pH

The curves represent various initial concentrations of xylocaine and of procaine hydrochloride (C_0) in mM at 25 C

Carbon dioxide-bicarbonate buffer.

The amount of bicarbonate and carbon dioxide which must be added to the solution to obtain a certain pH can be calculated from the HENDERSON-HASSELBALCH equation

$$pH = pk' + \log \frac{C_{HCO_3^-}}{C_{CO_2}} \tag{11}$$

with pk' as the first ionization constant of the carbonic acid in water. According to HARNED and DAVIS (1943) this is 6.326 at 30°C. The pk' increases by 0.005 per degree fall in temperature. CO_2 and HCO_3^- concentrations in the equation are expressed in M.

The carbon dioxide pressure can be expressed in M by the following expression

$$CO_2(M) = \frac{P \cdot \alpha \cdot n \cdot 1000}{760 \cdot 22.400 \cdot 100} \tag{12}$$

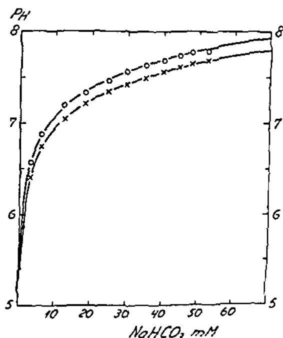


Fig. 4. pH of the carbon dioxide – bicarbonate buffer as a function of sodium bicarbonate concentration when the solution is saturated with 5 per cent CO_2.

○ experimental values in distilled water

× experimental values for Ringer's solution

The curves were calculated from the Henderson-Hasselbalch equation for a watery solution and for a solution with the ionic strength of Ringer's.

Ordinate: pH.

Abscissa: concentrations of $NaHCO_3$ in mM.

when P is atmospheric pressure in mm Hg n is the per cent content of CO_2 in the air mixture α is the HENRY s law constant for carbon dioxide in water (0 87 at 20°C, HARNED and DAVIS 1943)

Figure 4 shows how much bicarbonate had to be added to 1 litre of water saturated with 5 per cent carbon dioxide at 760 mm Hg and at 20°C to obtain pH values between 5 and 7 8 Experiments with distilled water and buffer showed agreement with the values calculated from the HENDERSON-HASSELBALCH equation

In experiments with Ringer s solution it was found that 40–46 per cent more bicarbonate must be added to obtain the same pH The reason for this is the effect of the ionic strength of the Ringer s salts To compensate the theoretical values with respect to ionic strength the dissociation constant k_s must be used instead of the thermodynamic constant K_a

k_s may be calculated from the following expression

$$pk_s = pK_a + \frac{0\,5\,(z_s^2 - z_b^2)\sqrt{\mu}}{1 + \sqrt{\mu}} \tag{13}$$

where z_s and z_b are the valencies of acid and base and μ is the ionic strength of the solution Since $z_s = 0$ and $z_b = -1$, μ is 0 134 and pK_a at 30°C is 6 33 pk_s is 6 20 at 30°C At 20°C pk_s is 6 25 Calculating the amounts of bicarbonate with this dissociation constant a relationship was obtained which corresponded closely to the experimental data (Figure 4)

At pH 7 it was necessary to add 11 mM/l bicarbonate corresponding to 22 m-equiv At higher pH the amount to be added increased sharply Thus at pH 7 7 110 m-equiv bicarbonate had to be added or 44 per cent of the total osmotic pressure of the Ringer s solution When pH exceeded 7, so much bicarbonate had to be added that the solubility product of calcium carbonate was exceeded and calcium ions were thrown out of the solution The use of 3 per cent carbon dioxide instead of 5 per cent reduced these difficulties considerably

Phosphate buffer.

FEHRENBERG (1948) indicates that phosphate buffer up to 10 mM does not alter the sensitivity of nerves to local anesthetics Phosphate buffer in higher concentrations causes the nerves to be anesthetized more readily This is perhaps due to the fact that free calcium ions, which are necessary to maintain nerve stability, are bound as insoluble calcium phosphate

Phosphate buffer is formed as



$$NaOH + NaH_2PO_4 \rightleftharpoons Na_2HPO_4 + H_2O \tag{14}$$

The concentration of NaH_2PO_4 used was 6.6 mM (LORENTE DE NO 1947). To calculate the amount of sodium hydroxide to be added to obtain a desired pH in phosphate-buffered Ringer's solution the dissociation constant k_s at the ionic strength of the solution had to be known. Since the solution in question was relatively concentrated, the dissociation constant could be calculated from the expression of COHN (1927)

$$pk_s = pK_a - \frac{1.5\sqrt{\mu}}{1 + 1.65\sqrt{\mu}} \tag{15}$$

Cohn (1927) determined pK_a to be 7.16. Since the ionic strength is 0.131, $pk_s = 6.82$.

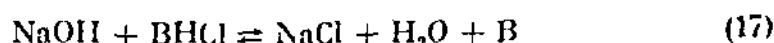
With various amounts of sodium hydroxide the pH could be calculated from

$$\text{pH} = 6.82 + \log \frac{C_{NaOH}}{C_{NaH_2PO_4} - C_{NaOH}} \tag{16}$$

where the concentration of sodium hydroxide (C_{NaOH}) equals the concentration of Na_2HPO_4 ($C_{NaH_2PO_4}$).

Figure 5 shows the agreement between the theoretical curve and the experimental values.

Sodium hydroxide added to xylocaine or procaine Ringer's solution combined partly with NaH_2PO_4 and partly with xylocaine or procaine hydrochloride as follows



$$NaOH + BHCl \rightleftharpoons NaCl + H_2O + B \tag{17}$$

where B is the uncharged xylocaine or procaine base, i.e. the amount of B formed was equal to the amount of sodium hydroxide bound. The value of B at various pH values was calculated from equation (1) (see the curves in Fig. 3). At the same time the amount of sodium hydroxide bound by the anesthetic was obtained. The total amount of sodium hydroxide required to obtain a certain pH in a phosphate buffered xylocaine or procaine Ringer's solution of a given concentration of hydrochloride could then be calculated.

Preparation of Ringer's and anesthetic solutions of different concentrations and pH.

It was requisite that all solutions, Ringer's as well as anesthetic solutions, be isotonic and with an osmotic pressure corresponding to 219 m-equiv. In addition, all solutions had to contain concentrations of potassium, calcium

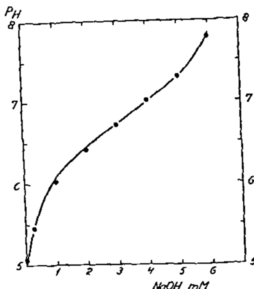


Fig 5 pH of the phosphate buffer in Ringer s solution as a function of the concentration of NaOH for a solution containing 6 6 mM NaH_2PO_4 and with an ionic strength of 0 134
● experimental values with phosphate buffer in Ringer's solution
The solid line is calculated with a pK_2 of 6 82
Ordinate pH
Abscissa concentration of NaOH in mM

and glucose as in normal frog Ringer s solution, as well as the reduced sodium concentration of 75 mM

In order to fulfill these demands and to be able to vary the concentration of anesthetic between 0 and 40 mM and to vary the pH of the buffered solutions between 5 5 and 7 8, 6 different stock solutions were prepared and mixed in suitable proportions to give a solution with desired properties

In appendix 1 the stock solutions are described in detail and the proportions in which they were mixed to obtain given Ringer s or anesthetic solutions

3

CHAPTER 3

MEASUREMENT OF THE AMPLITUDE OF THE ACTION POTENTIAL AND OF THE CONDUCTION VELOCITY OF THE NERVE IMPULSE

CONDITIONS INFLUENCING THE NERVE ACTION POTENTIAL AND ITS CONDUCTION VELOCITY

In this chapter an account is given of the factors which affect action potential amplitude and conduction velocity. Some of these are well known from the literature (temperature, carbon dioxide tension, pH). Others have not previously been investigated in detail, such as the distance and the resistance between the recording electrodes and the degree of stretch of nerve. With regard to the influence of temperature, carbon dioxide tension and pH only few experiments were carried out to ensure that the experimental arrangement allowed the results reported by others to be reproduced. The influence of distance and resistance between the recording electrodes and stretch of the nerve were studied in greater detail.

The stimuli applied to the nerve had a duration of 0.15 msec and a strength 2–3 times the strength which evoked an action potential of maximum amplitude. According to GASSER and ERLANGER (1937) such a stimulus activates α, β and γ fibers of the A group but not B and C fibers. According to GASSER and ERLANGER (1937) the threshold of B fibers is 33 and of C fibers is 100 times the threshold of α fibers. With the time scale used the action potentials of B and C fibers would not appear on the oscilloscope trace.

The nerve impulse was led off with two electrodes on the nerve, each recording a monophasic potential. Since the distance between the recording electrodes was less than the segment occupied by the monophasic potential, the difference between the two monophasic potentials was recorded as a diphasic potential.

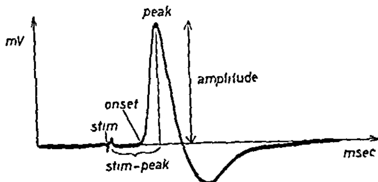


Fig 6 The definition of action potential amplitude and of measuring points for the determination of conduction velocity

Definitions.

Action potential amplitude

The action potential amplitude was measured in mV from the base line to the maximal negative deflection (Fig 6) The amplitude measured on ten successive action potentials recorded from the same nerve showed a mean error of $\pm$ 0 2 per cent The mean amplitude measured proximally to the test vessel on 63 different unanesthetized nerves was 5 88 mV $\pm$ 3 8 per cent

With a noise level of 2μV r m s the smallest action potential which could be measured was 10–30 μV in amplitude, i e 0 2–0 5 per cent of the amplitude before anesthesia To estimate how many nerve fibers contribute to this potential the number of nerve fibers was counted in a cross section of the nerve near the stimulating electrodes (Table 14) About 1300 large fibers with myelin sheath were found Assuming that each fiber contributes equally to the total action potential, an action potential amounting to 0 2–0 5 per cent of the initial amplitude arises from 3–6 nerve fibers That there were only few nerve fibers active when the smallest recorded action potential was obtained is also appearent from experiments on single nerve fibers (ADRIAN and BRONK 1928, HERTZ 1947) With leading-off conditions similar to those in the present experiments an amplitude of approximately 20–50 μV was found

Action potential conduction velocity

The conduction velocity was calculated in m/sec from the time of impulse conduction between the two sets of recording electrodes and the distance between them The point on the action potential used for the determination

Chapter 3

MEASUREMENT OF THE AMPLITUDE OF THE ACTION POTENTIAL AND OF THE CONDUCTION VELOCITY OF THE NERVE IMPULSE

CONDITIONS INFLUENCING THE NERVE ACTION POTENTIAL AND ITS CONDUCTION VELOCITY

In this chapter an account is given of the factors which affect action potential amplitude and conduction velocity Some of these are well known from the literature (temperature, carbon dioxide tension, pH) Others have not previously been investigated in detail, such as the distance and the resistance between the recording electrodes and the degree of stretch of nerve With regard to the influence of temperature, carbon dioxide tension and pH only few experiments were carried out to ensure that the experimental arrangement allowed the results reported by others to be reproduced The influence of distance and resistance between the recording electrodes and stretch of the nerve were studied in greater detail

The stimuli applied to the nerve had a duration of 0 15 msec and a strength 2 3 times the strength which evoked an action potential of maximum amplitude According to GASSER and ERLANGER (1937) such a stimulus activates α β and γ fibers of the A group but not B and C fibers According to GASSER and ERLANGER (1937) the threshold of B fibers is 53 3 and of C fibers is 100 times the threshold of α fibers With the time scale used the action potentials of B and C fibers would not appear on the oscilloscope trace

The nerve impulse was led off with two electrodes on the nerve each *recording a monophasic potential* Since the distance between the recording electrodes was less than the segment occupied by the monophasic potential, the difference between the two monophasic potentials was recorded as a diphasic potential

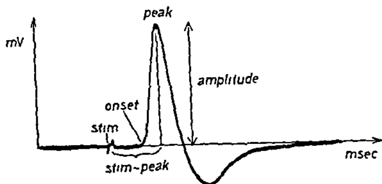


Fig 6 The definition of action potential amplitude and of measuring points for the determination of conduction velocity

Definitions

Action potential amplitude

The action potential amplitude was measured in mV from the base line to the maximal negative deflection (Fig 6) The amplitude measured on ten successive action potentials recorded from the same nerve showed a mean error of ± 0 2 per cent The mean amplitude measured proximally to the test vessel on 63 different unanesthetized nerves was 5 88 *mV* ± 3 8 per cent

With a noise level of 2μV r m s the smallest action potential which could be measured was 10–30 μV in amplitude, i e 0 2–0 5 per cent of the amplitude before anesthesia To estimate how many nerve fibers contribute to this potential the number of nerve fibers was counted in a cross section of the nerve near the stimulating electrodes (Table 14) About 1300 large fibers with myelin sheath were found Assuming that each fiber contributes equally to the total action potential an action potential amounting to 0 2–0 5 per cent of the initial amplitude arises from 3–6 nerve fibers That there were only few nerve fibers active when the smallest recorded action potential was obtained is also appearent from experiments on single nerve fibers (ADRIAN and BRONK 1928 HERTZ 1947) With leading off conditions similar to those in the present experiments an amplitude of approximately 20–50 μV was found

Action potential conduction velocity

The conduction velocity was calculated in m/sec from the time of impulse conduction between the two sets of recording electrodes and the distance between them The point on the action potential used for the determination

3*

of conduction velocity was the peak of the potential (Fig 6) The time interval between the peaks of the pre- and postanesthetically recorded potentials represented the conduction time over the section of nerve between electrodes L_{I_1} and L_{II_1} (Fig. 1) There were 7 mm between electrodes L_{I_1} and L_{I_2} in air and 32 mm between electrodes L_{I_2} and L_{II_2} in the anesthetic solution. Since the main object was to measure conduction velocity over the anesthetized section of nerve, the total conduction time had to be corrected for the conduction time over the 7 mm of nerve in air. To this purpose the conduction velocity was determined with the whole nerve in air before the anesthetic experiment

The conduction velocity measured 10 times in the same nerve showed a mean error of ± 0.2 per cent. The mean conduction velocity in Ringer's solution at 22°C for 10 different nerves was 31 3 m/sec ± 2.1 per cent

Amplitude and conduction velocity of the action potential measured on a whole nerve in relation to measurements on single fibers.

The action potential recorded from a whole nerve is the sum of the action potentials of the single fibers Since these conduct at various velocities there results a temporal dispersion along the nerve GASSER and ERLANGER (1937) analyzed the shape of the *action potential of whole nerve as a function of* the distance from the stimulating point and interpreted the shape in terms of the distribution of fiber diameters, conduction velocities and spike am*plitudes*

Amplitude of the action potential

Under the conditions of the experiments reported here temporal dispersion and differences in the external shunt resulted in a higher amplitude of the action potential measured at L_I (preanesthetic recording) than recorded at L_{II} (postanesthetic recording) The amplitude recorded at L_I was higher when the nerve was stimulated proximally than when it was stimulated distally This was due to the fact that fibers branch off*) from the main nerve between the two recording points When the stimulating point was proximal the action potentials of these fibers were recorded at L_I but not at L_{II} When the stimulation was distal the same number of fibers was recorded from both points since all stimulated fibers extended throughout the nerve For this reason the nerve was always stimulated distally in the experiments concerning anesthesia

*) Ramus profundus anterior and posterior leave the sciatic nerve 16 mm proximal to electrode L_{I_1} when the nerve is stimulated at the distal end

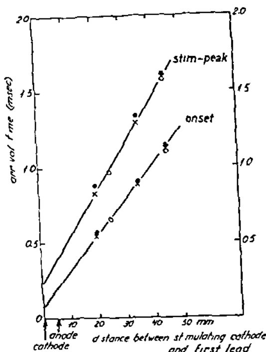


Fig 7 Conduction time of the action potential over various distances of the nerve (three experiments ● ○ ×)

Ordinate time in msec measured from the stimulation artefact to the onset and to the peak of the potential

Abscissa distance on the nerve in mm measured between the stimulating cathode and the first recording electrode

Conduction velocity of the action potential

If the onset of the action potential is used for the determination of conduction velocity temporal dispersion of the action potential does not distort the measurement since the onset always corresponded to the most rapidly conducted impulses (see Fig 6) Using the peak of the action potential as the point to be measured the change in the shape of the action potential during propagation caused a shift in the point to be measured which was the greater the larger the distance between the stimulation and recording points The peak of the action potential recorded from the postanesthetic section occurred therefore later than in the preanesthetic section so that the conduction time measured was longer (see Fig 7) The peak was however, easier to identify than the onset especially when the amplitude was low due to anesthetic effect Since the object of the anesthetic experiments was to

determine *changes* in conduction velocity the peak of the action potential could be used as long as the distance between stimulating and recording electrodes was the same in the experiments in which conduction velocities were to be compared

Action potential with various placements of the stimulating cathode and anode.

Action potential amplitude

When the anode of the stimulating electrode pair was placed closest to the recording electrode, the action potential amplitude was about 20 per cent lower than when the anode was placed distally This difference in amplitude was due to anode block of some of the fibers between the cathode and the recording electrodes The proximal electrode of the stimulating pair was therefore the cathode in the anesthetic experiments (see Fig 2)

Conduction velocity of the action potential

Stimulating a nerve with the cathode as the distal or as the proximal stimulating electrode, and measuring the conduction time from the stimulus artefact to the action potential, RUSHTON (1949) found that the point of initiation of the nerve impulse did not coincide with the cathode but lay some 3 mm from it extrapolarly The determination of conduction time from the interval between stimulus artefact and action potential is unsuitable for other reasons

1) when the stimulus is of high intensity the point of stimulation may be still further from the cathode
2) stimulation can only take place at the site of a Ranvier s node (TASAKI 1953) Since the nodes occur at intervals of 0 2–1 mm and are displaced for the single fibers, the site of stimulation extends over a distance of up to 1 mm along the nerve
3) the latency time, an unknown quantity, occupies some of the time between stimulus and onset of the recorded action potential

The unsuitability of the stimulus artefact as point of reference is illustrated in Figure 7 Extrapolation to the abscissa of the arrival times as measured to the onsets and to the peaks of the action potentials indicated that stimulation of the nerve occurred extrapolarly and not at the cathode

Changes in the action potential with various distances between the recording electrodes.

Since the diphasic action potential represents the difference between the two monophasic potentials recorded at the two recording electrodes the

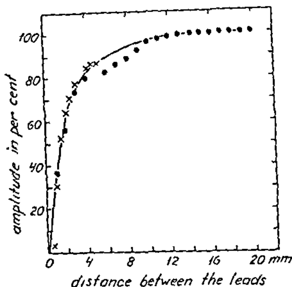


Fig 8 Amplitude of the nerve action potential as a function of the distance between recording electrodes (two experiments ×, ●)
Ordinate amplitude in per cent of the maximum value
Abscissa distance between electrodes in mm

amplitude and shape of the action potential depend on the distance between the recording electrodes In two experiments this distance was varied between one half and 20 mm and the following changes in amplitude and conduction velocity were found

Action potential amplitude

Figure 8 demonstrates that the amplitude increased with increasing distance between the recording electrodes up to a distance of 14 mm Figure 9 shows action potentials recorded from electrodes at a distance of fourteen and of one mm from each other, as well as the reconstructed monophasic potentials recorded at each of the two recording electrodes (LORENTE DE NO 1947 and HAKANSSON 1957)

When the distance between the recording electrodes was 14 mm the monophasic potential recorded at the second recording electrode had just begun when the amplitude of the monophasic potential recorded at the first electrode was maximal and it did not diminish the front of the diphasic potential The amplitude of the diphasic potential was equal to that of the monophasic potential

At electrode distances less than 14 mm a decrease in amplitude occurred

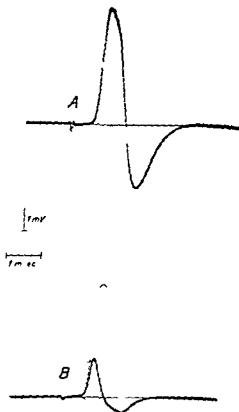


Fig 9 Diphasic action potentials recorded with (A) 14 mm and (B) 1 mm between the recording electrodes The stippled lines are the monophasic potentials recorded at each lead reconstructed from the diphasic potentials

since the second monophasic potential had its onset during the front of the potential recorded at the first electrode as shown for example in Figure 9 The distance of 11 mm corresponding to the front of the monophasic potential is confirmed by measurement of potentials illustrated by Lorente de Nó (1947)

To obtain amplitudes independent of minor variations in distance between the recording electrodes requires that they be separated by more than 11 mm In view of the limited nerve length available however the recording electrodes of a pair were 7 mm apart According to Figure 8 a variation of 1 mm from this inter electrode distance would cause a change in amplitude of 2 3 per cent

Conduction velocity of the action potential

The conduction velocity was independent of the distance between the recording electrodes when the time interval between the onset of the action potentials was measured. Measuring the time interval between the peaks of the potentials the conduction time was independent of the distance between the electrode pair when this was greater than 6 mm. With smaller inter electrode distances the conduction time was shorter.

Alterations of the action potential occurring with changes in the resistance between the recording electrodes

Action potential amplitude

The resistance between the recording electrodes might alter in the course of an experiment as for example when the layer of fluid surrounding the nerve evaporated. It is known that the amplitude and conduction velocity of the nerve impulse are dependent on the resistance of the medium surrounding the nerve (HODGKIN 1939). It was therefore of interest to ascertain whether a change in resistance between the recording electrodes could influence the amplitude and conduction velocity of the action potential with the set up used. To this purpose a nerve was laid in the open chamber so that the surrounding fluid layer could evaporate. Figures 10 A, B show examples of the relationship between the resistance between the recording electrodes and the amplitude and conduction velocity of the action potential. The resistance increased rectilinearly in the course of 50 minutes from 9.2×10^3 to 10×10^3 Ohm. The action potential amplitude increased at first proportionally with the resistance and reached a maximum in 30 minutes. Denoting the resistance of the nerve between the recording electrodes R_n, the potential difference over it V_n, the resistance in the nerve including the fluid shunt R and the potential difference over it V, the rectilinear portion of the curve is given by

$$\frac{V_n}{V} = \frac{R_n}{R}$$

During the time in which the amplitude increased rectilinearly with resistance the *increase was due solely to a change in the fluid shunt* and not in the size of the monophasic potential. In other words the number of active fibers and the amplitude of each single fiber's action potential were unchanged. The nerve was allowed to dry both between stimulating and recording electrodes and between the two recording electrodes. The increase in amplitude could be explained by the change in resistance between the

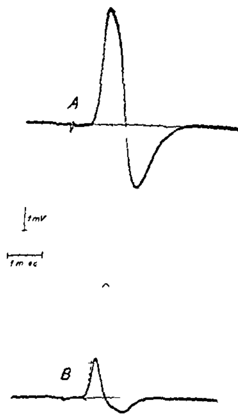


Fig. 9. Diphasic action potentials recorded with (*A*) 14 mm and (*B*) 1 mm between the recording electrodes. The stippled lines are the monophasic potentials recorded at each lead reconstructed from the diphasic potentials.

since the second monophasic potential had its onset during the front of the potential recorded at the first electrode as shown for example in Figure 9. The distance of 11 mm corresponding to the front of the monophasic potential is confirmed by measurement of potentials illustrated by LORENTE DE NÓ (1947).

To obtain amplitudes independent of minor variations in distance between the recording electrodes requires that they be separated by more than 11 mm. In view of the limited nerve length available, however, the recording electrodes of a pair were 7 mm apart. According to Figure 8 a variation of 1 mm from this inter-electrode distance would cause a change in amplitude of 2.3 per cent.

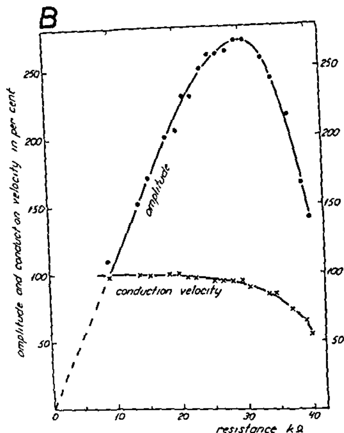


Fig 10

B Action potential amplitude and conduction velocity as a function of the increasing resistance between recording electrodes with the nerve in dry air (cf A)

Ordinate action potential amplitude and conduction velocity in per cent of the values with the nerve in Ringer's

Abscissa resistance of the nerve plus fluid shunt in kOhm measured between the two recording electrodes

of the evaporation experiments and not increase until the fluid layer around the nerve was saturated with ions i e when the specific resistance was constant However the amplitude increased from the onset of the evaporation period probably because the ions diffused into the nerve as their concentration in the fluid increased so the specific resistance remained constant during the whole period of evaporation and the resistance depended solely on the thickness of the fluid layer After approximately 25–30 minutes of drying the amplitude fell and after 75 minutes it was close to zero Since the

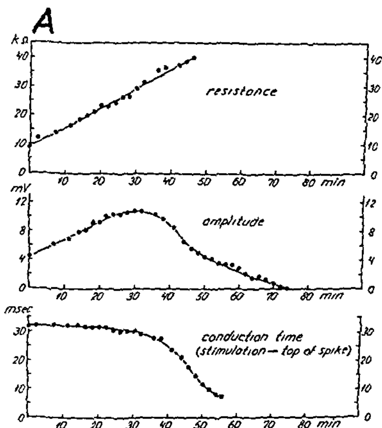


Fig 10 The influence of the increasing resistance between the recording electrodes
A Resistance between recording electrodes, action potential amplitude and conduction time in the same nerve as a function of time for its exposure to dry air

Upper ordinate	resistance of the nerve plus the fluid shunt between recording electrodes (kOhm)
Middle ordinate	action potential amplitude in mV
Lower ordinate	conduction time measured from the stimulus artefact to the peak of the potential in msec
Abscissa	time the nerve has been exposed to dry air in minutes

recording electrodes and was not due to the change in the fluid shunt between stimulating and recording electrodes as demonstrated by the following experiment

The resistance between the recording electrodes was kept constant the external resistance between the stimulating and recording electrodes was diminished from 92.4×10^3 Ohm to 21.1×10^3 Ohm by placing a strip of filter paper moistened with Ringer's solution on the nerve The amplitude measured without external shunt was 5.1 mV and with the filter paper 5.3 mV in other words identical within the accuracy of measurement

It was to be expected that the amplitude be unchanged at the beginning

trodes the resistance could be deduced from changes in amplitude at the preanesthetic electrodes An example of this is described on page 56

Alterations of the action potential by stretching the nerve.

Since the nerve was slightly stretched between the electrodes in the chamber it was determined whether such stretch could alter the amplitude and conduction velocity of the action potential

In a series of experiments the nerve was submitted to various degrees of stretch At each degree of stretch the action potential and the resistance between two recording electrodes were measured The stretch of the nerve was expressed as per cent of the initial length

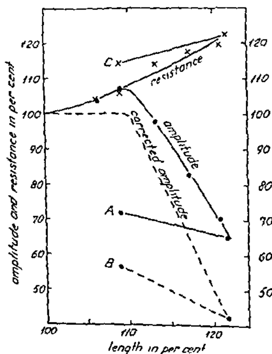


Fig. 11 Action potential amplitude and resistance between two recording electrodes as a function of stretch of the nerve

Ordinate amplitude and resistance in per cent of values measured on the unstretched nerve

Abscissa length of the nerve in per cent of that of the unstretched nerve

Points A and C were obtained when the nerve was allowed to relax after maximum stretch The broken lines represent the amplitude corrected for the increase in resistance (B is the corrected value of A)

condition was not reversible when the nerve was again placed in Ringer's solution, the explanation must be that the nerve fibers were damaged and ceased to conduct

Conduction velocity of the action potential

In the period when the amplitude increased rectilinearly as a function of the increase in resistance between the recording electrodes the conduction velocity did not change (Figs 10 A, B) This was also shown in experiments where the resistance between stimulating and recording electrodes was decreased by means of an extra shunt, the conduction velocity was unchanged when filter paper soaked in Ringer's solution was placed on the nerve

In the case of single fibers RUSHTON (1937) deduced theoretically, and his deduction was confirmed experimentally by HODGKIN (1939) and KATZ (1947), that the conduction velocity is inversely proportional to the square root of the sum of the inner and outer resistance, so that conduction velocity falls with increased outer resistance That this did not obtain in the case of whole nerve can be explained by the fact that drying did not alter the resistance in the interfibrillar fluid When the amplitude began to fall as the evaporation was nearly complete, the conduction velocity also fell towards zero The fall in conduction velocity might be explained by the disappearance of the interfibrillar fluid, but if this were so the amplitude should increase on account of the increased outer resistance around the single fibers As mentioned above the amplitude decreased, probably because of damage to the single fibers, causing them to lose potassium and their membrane potential to be diminished This, too, would involve a reduction in conduction velocity (LORENTE DE NO 1947)

Conclusion: Since the fluid shunt surrounding a nerve is dependent on the humidity of the surrounding air, variations in humidity can cause changes in resistance between the recording electrodes and thereby cause changes in the amplitude of the action potential During long lasting anesthetic experiments it was therefore necessary to measure the resistance at frequent intervals so that the amplitude could be corrected for a change in resistance The conduction velocity of the action potential did not decrease until the resistance in the fluid shunt increased to more than three times its initial value A correction of conduction velocity was therefore not requisite in the range of resistance changes which occurred during the experimental conditions

In addition to direct resistance measurements between the recording elec-

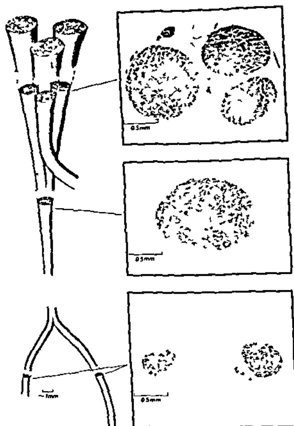


Fig. 12 Histological section through a frog nerve (osmium stain)
Above at the site of the post anesthetic recording electrodes in nerves without sheath (8th 9th and 10th spinal nerves)
Middle at the site of the post anesthetic recording electrodes in nerves with sheath (sciatic nerve)
Below at the point of stimulation (tibial and peroneal nerves)

were placed on a segment of the nerve which had not been stripped of its sheath The postanesthetic recording electrodes were therefore placed on the three spinal nerves in experiments on nerves without sheath The resistance between the recording electrodes in these experiments was smaller than in experiments on nerves with intact sheath, where the recording electrodes were placed on the sciatic nerve This is illustrated on the histological section through the nerve at the two recording sites (Figure 12), where it is seen that the cross sectional area in experiments "without sheath" was 1 8 times greater than in experiments with intact sheath The smaller resistance

The action potential amplitude and the resistance between the recording electrodes increased 7–8 per cent with a 10 per cent stretch of the nerve (Fig 11) Since the amplitude increased proportionately with the resistance between the recording electrodes the increase in amplitude found on stretching the nerve was probably due solely to the increased resistance resulting from the stretch

When the nerve was stretched more than 10 per cent the amplitude fell while the resistance continued to rise This is assumed to be due to the fact that some of the nerve fibers were blocked, resulting in a decrease in the amplitude of the whole nerve action potential On account of the progressive increase in resistance between the recording electrodes, this decrease was still more pronounced than it appeared from the action potential amplitude Figure 11 shows the reduction in amplitude of the nerve impulse due to blocking of single nerve fibers because of stretch when corrected for the increase in resistance

If the nerve was allowed to relax after a stretch of about 22 per cent, the length returned to 109 per cent, the resistance to 114 5 per cent, and the corrected amplitude increased to no more than 55 per cent of the initial value (point B, Fig 11) The decrease in amplitude with stretch is therefore only partially reversible The irreversible decrease in amplitude may be due to tear of some of the nerve fibers

The conduction velocity of the action potential was unchanged for stretches of the nerve up to 20 per cent Thereafter it fell abruptly by about 10 per cent When the nerve was allowed to relax the conduction velocity increased slightly, but did not return entirely to normal That the conduction velocity was constant over such large degrees of stretch is in agreement with the findings of BULLOCK et al (1950) who investigated conduction velocity during stretch of single giant nerve fibers of Lumbricus and Loligo

Conclusion: The nerve could be stretched by at most 10 per cent without damaging the fibers With higher degrees of stretch the action potential amplitude fell due to fiber damage With stretch of less than 10 per cent the amplitude could be corrected for the increased resistance between the recording electrodes, since the amplitude change with stretch of the nerve was proportional to the change in resistance

The action potential amplitude in experiments on nerves without sheath.

The dissection of the sheath was carried out distal from the union of the 8th, 9th and 10th spinal nerve To obtain as similar recording conditions as possible in experiments with and without sheath the recording electrodes

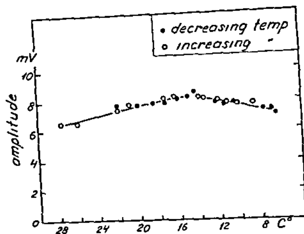


Fig 13 Action potential amplitude as a function of temperature

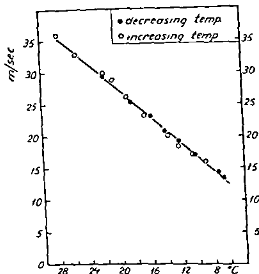


Fig 14 Conduction velocity of the action potential as a function of temperature (determined for the nerve illustrated in Fig 13)

Dependence of the nerve action potential on pH.

There was no change in action potential amplitude or conduction velocity when the pH of the Ringer's solution in the test chamber was varied between 5 8 and 7 7 This is in agreement with investigations on single frog nerve fibers (HERTZ 1947) Similarly LORENTE DE NO (1947) found for whole frog

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between the recording electrodes in experiments "without sheath" results in correspondingly lower action potential amplitudes compared with experiments on nerves with sheath.

The mean action potential amplitude in Ringer's solution for 17 nerves "without sheath" was 2.9 mV ± 8.6 per cent (Table 9) in comparison with 5.88 mV ± 3.8 per cent for 63 nerves with sheath.

The effect of temperature and of carbon dioxide tension on the amplitude and conduction velocity of the action potential.

Action potential amplitude

LORENTE DE NÓ (1947) found that the action potential amplitude was maximal at a temperature of 10–15°C, depending on the temperature to which the frog was accustomed. For temperatures above and below this value the amplitude fell slightly. I have confirmed these findings in the experiment shown in Fig. 13. The amplitude was maximum at 15°C and was about 10 per cent less at 22° and 8°C. Thus, within the temperature range which obtained during an anesthesia experiment the changes in amplitude due to temperature changes were so small that they could be disregarded.

According to LORENTE DE NÓ (1947) it is necessary that the carbon dioxide concentration be 3–5 per cent for normal functioning of the nerve. Carbon dioxide is said to have a specific effect on nerve metabolism. Lack of carbon dioxide results in a decrease in the resting membrane potential and therefore in the amplitude of the action potential. RUDOLPH (1953) found a 20 per cent greater amplitude in the presence of 5 per cent carbon dioxide than in its absence. With the nerve in a humid atmosphere of 97 per cent oxygen and 3 per cent carbon dioxide I found no change in the action potential amplitude over as long a period as 5 hours.

Conduction velocity of the action potential

The conduction velocity falls with decreasing temperature. It is well known that the conduction velocity changes relatively more with temperature than the amplitude. Values of Q_{10} for the dependence of the conduction velocity on temperature as determined by different investigators are given in Table 1. GASSER's (1931) findings were confirmed in the experiment shown in Figure 14, the Q_{10} being 1.71 at 8° to 18°C and 1.12 at 18–24°C. The velocity fell rectilinearly with decreasing temperature with a slope corresponding to 1 m sec. per degree centigrade.

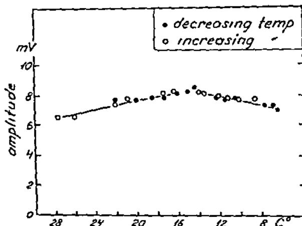


Fig 13 Action potential amplitude as a function of temperature

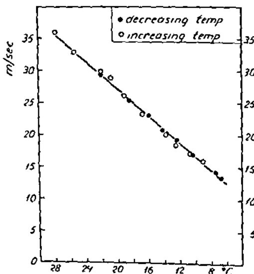


Fig 14 Conduction velocity of the action potential as a function of temperature (determined for the nerve illustrated in Fig 13)

Dependence of the nerve action potential on pH.

There was no change in action potential amplitude or conduction velocity when the pH of the Ringer s solution in the test chamber was varied between 5 8 and 7 7 This is in agreement with investigations on single frog nerve fibers (Huxtz 1947) Similarly Lorente de No (1947) found for whole frog

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Table 1.

Dependence of conduction velocity on temperature

author	nerve	temperature range °C	Q_{10}
MAXWELL, 1907	pedal nerve (*Ariolimax columbianus*)	10–20	1.55
LUCAS, 1908	sciatic, tibial and sural nerves (*Rana esculenta*)	8–18	1.79
GASSER, 1931	sciatic nerve (green frogs)	8–18 18–21	2.0 1.7

nerve that the activity was not altered by changes in pH between 5.5 and 8. Below pH 3 impulse conduction is blocked. With pH values above 8 spontaneous impulses appear due to the precipitation of calcium (LEHMANN 1937b).

A change in pH is however accompanied by a change in the threshold to stimulation. ADRIAN (1920) found for frog nerve that the threshold to stimulation was 36 per cent higher at pH 5.5 than at pH 9.6. LEHMANN (1937a) found that the threshold in cat nerve rose rectilinearly by a factor of four from pH 8 to 7.2. The fact that the nerve action potential is unchanged with variations in pH may be explained by the fact that the nerve impulse is in any case large enough to depolarize the adjacent not yet activated section of nerve membrane in spite of its elevated threshold.

Dependence of action potential amplitude on the outer sodium concentration.

To obtain isotonic anesthetic solutions the sodium concentration in the Ringer's and anesthetic solutions was decreased from 115 mM (the normal sodium content for frog's plasma) to 75 mM. It was therefore necessary to determine whether the action potential amplitude varied with the outer sodium concentration. Allowing a whole frog nerve to conduct in normal Ringer's and then for 1½ hours in Ringer's solution with 75 mM sodium, no change in amplitude occurred.

The action potential amplitude of the single fiber depends on the outer sodium concentration, the overshoot being proportional with the outer sodium concentration (HUXLEY and STAMPFLI 1951). With 75 mM sodium

in the bathing fluid or 64 per cent of the concentration in normal Ringer's, the overshoot is diminished by about 10 mV (HUXLEY and STAMPFLI 1951). For an action potential amplitude of 116 mV in normal Ringer's this decrease in amplitude amounts to 9 per cent.

The reason that I found no reduction in experiments on whole nerve is probably that the diminished external sodium concentration does not infiltrate the single fibers in the time over which the experiments extend. A whole nerve can conduct for many hours in a sodium free Ringer's solution without impairment.

FACTORS OF SPECIAL IMPORTANCE DURING LOCAL ANESTHESIA

The influence of the length of the anesthetized portion of nerve.

Experiments with 20 mm of nerve exposed to a high concentration of the local anesthetic showed that the amplitude of the action potential as recorded from the postanesthetic section did not diminish to zero. There persisted a potential of 0.1–0.15 mV corresponding to 1.2 per cent of the amplitude in Ringer's even after the nerve had been in the anesthetic solution for 50 minutes. The diminution in amplitude to 1.2 per cent occurred in the course of about 6 minutes. To investigate whether the persisting small potential originated from electrotonic conduction over the anesthetized section the following experiment was performed.

The action potential and the electrotonically conducted potential are affected oppositely by changes in the resistance, the action potential amplitude increasing with increased outer resistance.

The outer resistance of the anesthetized section of nerve was changed by placing the nerve first in air and then in Ringer's solution. Since the small persisting potential was about three times greater when the nerve was in Ringer's solution than in air it was an electrotonically conducted potential.

Increasing the length of the anesthetized section of nerve above 20 mm caused a decrease in the amplitude of the electrotonically conducted potential (Fig. 15). At 31 mm it could no longer be discriminated. As a result the experimental chamber was designed to allow 32 mm of nerve to be subjected to the anesthetic and in the subsequent experiments no electrotonic potential was recorded in the postanesthetic section.

The whole section of nerve in the experimental chamber was subjected to the same concentration of the local anesthetic, since the remaining part of the nerve was surrounded by humid air. It is assumed that conduction occurred with constant velocity over the part of the nerve exposed to the local anesthetic.

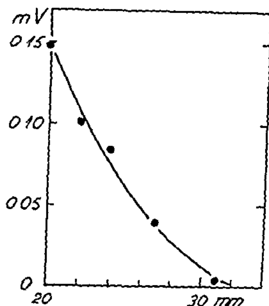


Fig 15 Size of the electrotonic potential recorded by the post anesthetic electrode as a function of the length of the nerve segment blocked by the local anesthetic
Ordinate electrotonic potential in mV
Abscissa length of the blocked segment of nerve in mm

LUCAS and coworkers (1917) contrary to this assumption interpreted their anesthetic experiments to indicate that the size and velocity as well as the threshold to stimulation of the nerve impulse diminished or showed decrement along the course of anesthetized nerve KATO (1924 1926) claimed on the other hand that in the single fiber either a maximal impulse could be elicited or no impulse at all and that the conduction velocity and threshold were constant along the nerve Immediately before the nerve was blocked both the size of the impulse and the velocity of its conduction diminished and the threshold to stimulation was elevated

According to KATO (1924, 1926) LUCAS and coworkers (1917) had neglected to consider the following circumstances

1) At the boundary between normal and anesthetized nerve a diffusion of the anesthetic occurs into the normal nerve and a diffusion of Ringer's solution into the anesthetized nerve In this way about 3 mm of nerve on either side of the boundary are subjected to various concentrations of anesthetic Since LUCAS anesthetized only 1 5 mm of nerve the conditions along the nerve were not constant That the conduction velocity amplitude and threshold varied along the anesthetized section could therefore not be taken as evidence for the existence of decrement in this section

2) With electrical stimulation the electrotonically conducted potential

influences the threshold KATO used mechanical stimulation and found that the threshold was constant along the nerve at the same degree of anesthesia

When only a short section of the nerve is anesthetized the electrotonic potential can excite the nerve beyond the blocked section ADRIAN (1912) found for example that the nerve impulse can jump over a section as long as 5 mm HODGKIN (1937) measured the spread of electrotonic currents over the blocked section About a tenth of the electrotonic current which spreads from an activated section of nerve is sufficient to activate neighbouring areas HODGKIN (1937) found a propagated potential when the blocked section of nerve was less than 7 8 mm An increased outer resistance diminishes the electrotonic spread and therefore also the length of the blocked section over which the action potential can jump HURYAMA (1911) states that the minimum length of nerve to be blocked is 10 mm to ensure an instantaneous effect of an anesthetic on a single fiber With smaller distances the blocking time is prolonged corresponding to the time it takes for the anesthetic to diffuse over the critical section of nerve

In the investigation reported here a 32 mm section of nerve was exposed to the local anesthetic to 1) prevent activation of the post-anesthetic section of the nerve by electrotonically spread potentials and to 2) prevent distortion of the post anesthetically recorded action potential by electrotonic spread

The action potential recorded from the preanesthetic portion of the nerve

This potential was used as point of reference when determining conduction velocity over the anesthetized section and as an indicator of the general condition of the nerve To be able to record conduction velocity solely over the section of nerve anesthetized (see however page 36) the proximal recording electrode L_{1_1} (see Fig 1) was in direct contact with the anesthetic fluid As a result the shape of the preanesthetic potential changed as anesthesia progressed (see below)

The amplitude of the pre-anesthetic action potential increased with increasing degrees of anesthesia and the action potential became monophasic when block was complete (see Fig 16) The increase of amplitude may be explained as follows (see also page 39)

Since the second electrode of the preanesthetic pair of recording electrodes was in contact with the anesthetic fluid the potential recorded by it disappeared resulting in an amplitude recorded solely at the first recording electrode This amplitude had the same size as the monophasic potential



Fig 16 Pre and post anesthetic action potentials with progressing local anesthesia of the nerve

The pre anesthetic potential (upper beam) becomes gradually monophasic and its amplitude increases The post anesthetic potential (lower beam) diminishes in amplitude with increasing anesthesia The conduction time from stimulus (S) to amplitude peak is gradually prolonged indicating a diminution in conduction velocity in the anesthetized section of the nerve (2 mm between recording electrodes)

(maximal amplitude), even if the distance between the recording electrodes was less than 11 mm With electrode distances greater than 11 mm no change in the preanesthetic potential amplitude occurred during anesthesia of the nerve The relationship between the initial and final amplitude of the action potential recorded preanesthetically as a function of electrode distance, is shown in Table 2

During anesthesia of the nerve the amplitude recorded at the preanesthetic electrode increased rectilinearly as the amplitude at the postanesthetic electrode diminished (Fig 17) Hence, a measure of the effect of the anesthetic on the nerve could be obtained from the amplitude recorded at the preanesthetic electrodes. Since, however, the amplitude varied by no

Table 2

Amplitude of the pre anesthetic diphasic action potential for various distances between the recording electrodes

electrode distance mm	amplitude in per cent	
	referred to the amplitude of the monophasic pre anesthetic potential of the blocked nerve*)	referred to the amplitude of the potential measured with an electrode distance exceeding 14 mm**)
1	35	36
2.5	65.5	69
5	81.4	86.5
7	95.1	92.5
14	100	100

*) block at electrode I_{1}

**) values from Fig 8

more than about 10 per cent with electrode distances of 7 mm, the uncertainty was large

By diminishing the distance between the preanesthetic recording electrodes the amplitude recorded from them varied more with anesthesia With a distance between electrodes of 1 mm the variation was about 65 per cent (Figs 9 17) In view of KATO s finding (1921) that the anesthetic diffuses along the nerve the nerve may with this short interelectrode distance be anesthetized at the first (peripheral) electrode as well causing a reduction in the amplitude of the potential recorded here With a 7 mm inter-electrode distance diffusion of the anesthetic along the nerve would affect the action potential amplitude only slightly

In view of the relationship between the pre-and postanesthetic potential amplitudes during anesthesia (Fig 17) the amplitude of the preanesthetic potential allows the results from the postanesthetic recording to be checked At the time when the latter had fallen to zero the amplitude at the preanesthetic recording must have increased to its maximum If the preanesthetic potential amplitude was less than maximum, the nerve had suffered damage If the amplitude of the preanesthetic potential was greater than its maximum the fluid layer around the nerve must have evaporated, resulting in



Fig 16 Pre and post anesthetic action potentials with progressing local anesthesia of the nerve

The pre anesthetic potential (upper beam) becomes gradually monophasic and its amplitude increases The post anesthetic potential (lower beam) diminishes in amplitude with increasing anesthesia The conduction time from stimulus (S) to amplitude peak is gradually prolonged indicating a diminution in conduction velocity in the anesthetized section of the nerve (2 mm between recording electrodes)

(maximal amplitude), even if the distance between the recording electrodes was less than 1½ mm With electrode distances greater than 1½ mm no change in the preanesthetic potential amplitude occurred during anesthesia of the nerve The relationship between the initial and final amplitude of the action potential recorded preanesthetically as a function of electrode distance, is shown in Table 2

During anesthesia of the nerve the amplitude recorded at the preanesthetic electrode increased rectilinearly as the amplitude at the postanesthetic electrode diminished (Fig 17) Hence, a measure of the effect of the anesthetic on the nerve could be obtained from the amplitude recorded at the preanesthetic electrodes Since, however, the amplitude varied by no

Conduction velocity of the action potential

The change in action potential shape at the preanesthetic recording electrodes during anesthesia of the nerve did not influence the onset of the potential The time from the point of stimulation to the peak of the potential was increased on account of the disappearance of the positive portion of the action potential recorded at the proximal recording electrode In experiment no 79 for example with recording electrodes 7 mm apart this time increased by 0 015 msec from the beginning of the experiment to full blockage of the nerve With a conduction velocity of 30 m/sec this corresponded to 0 45 mm of nerve When the conduction velocity was measured over 39 mm of nerve the error was 1 2 per cent

The action potential recorded from the postanesthetic portion of the nerve

The interelectrode distance was of no importance for the *amplitude* of the postanesthetic action potential during anesthesia of the nerve since the nerve was blocked at the first recording electrode

With respect to *conduction velocity* during anesthesia it is shown on page 119 that the nerve conducted at normal velocity when the impulse had passed the anesthetized section of nerve

EXPERIMENTAL METHOD ADOPTED FOR MEASUREMENT OF ACTION POTENTIAL AMPLITUDE AND CONDUCTION VELOCITY

On the basis of the considerations and experiments performed with respect to the influence of various factors on action potential amplitude and conduction velocity the following experimental method was adopted

1 The stimulus had a duration of 0 15 msec and an intensity 2–3 times threshold of maximum action potential amplitude in order to allow recording of action potentials from α β and γ fibers of the A group (see p 34)

2 The nerve was stimulated at its distal end so that all activated fibers passed uninterruptedly throughout the anesthetized section of nerve (see p 36)

3 The proximal stimulating electrode was used as cathode to avoid a reduction in amplitude on account of anodal block (see p 38)

4 The distance between the ground electrode and the closest recording electrode was more than 6 mm to avoid stimulus artefact (see p 20)

5 To ensure independence of amplitude from minor variations in distance between the recording electrodes an inter-electrode distance of 7 mm was used A variation of 1 mm from this inter-electrode distance resulted in a variation in amplitude of 2–3 per cent (see p 40)

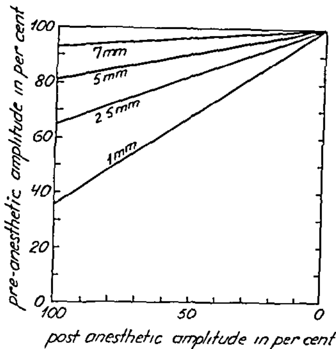


Fig 17 Increase in action potential amplitude at the pre anesthetic recording as anesthesia progresses for various distances between recording electrodes as indicated on the curves The amplitude of the post anesthetic potential indicates the degree of anesthesia Ordinate amplitude of the pre anesthetic potential in per cent of the final value Abscissa amplitude of the post anesthetic potential in per cent of the value in Ringer s

an increased resistance between the recording electrodes and an increased amplitude

From the amplitude of the preanesthetic potential it was possible to calculate changes in the resistance between the recording electrodes In experiment 1C2 for example the amplitude at the preanesthetic electrode was 14 7 mV at the beginning of the experiment with the nerve in Ringer s and with a 7 mm inter electrode distance At the stage of anesthesia when the amplitude of the postanesthetic potential was 50 per cent the preanesthetic potential amplitude was 18 25 mV This increase in amplitude could arise from partial blocking of the nerve at the proximal electrode of the preanesthetic electrode pair plus the effect of increase in resistance between the recording electrodes With the nerve in Ringer s the amplitude should be 93 per cent of its maximum value (Fig 17) when the electrodes were separated by 7 mm The maximum amplitude must therefore have been 15 8 mV When the postanesthetic amplitude was 50 per cent the preanesthetic amplitude according to Figure 17 should be 96 5 per cent of its maximum i e 15 3 mV The actually found value of 18 25 mV was therefore 19 per cent greater than to be expected The resistance between the preanesthetic recording electrodes was measured with the nerve in Ringer s to be 26 0 × 10³ and at 50 per cent amplitude to be 31 1 · 10³ Ohm i e the resistance increased 18 per cent and could account for the increase in amplitude of the preanesthetic potential (cf p 41)

Chapter 1

THE ANESTHETIC EFFECT OF XYLOCAINE AND PROCAINE ON PERIPHERAL NERVE

The purpose of the investigations described in this chapter was to investigate the time course of xylocaine and procaine anesthesia in a peripheral nerve. Considering that a local anesthetic must diffuse from the outer fluid into the nerve, the time course of anesthesia must be assumed to depend on the ratio between the minimum concentration and the outer concentration of the active component of the anesthetic. It is assumed that the active component is the uncharged base portion of the anesthetic (Gros 1910, Trevan and Boock 1927, Gardner, Semb and Graham 1931 and Ehrenberg 1948). However Skou (1954) using muscle contraction as indicator, found that the minimum concentration of procaine expressed as uncharged base increased with increasing pH, and for this reason he doubts that the base alone is the anesthetically active portion.

In a series of experiments the minimum concentration of xylocaine and procaine at various pH levels was determined with the action potential as indicator. In another series of experiments the time course of anesthesia with procaine and xylocaine was determined with various concentrations of base. To contribute to the question as to whether it is the uncharged base which is the active component, experiments have been compared in which the concentration of base was constant while pH differed, and when the nerve was subjected to the same hydrochloride concentration at increasing pH levels with corresponding increase in concentration of base and decrease in concentration of acid.

Finally, to investigate the importance of the nerve sheath for the time course of anesthesia, experiments were carried out on nerves without sheath.

RESULTS

Minimum concentration of xylocaine and procaine.

The minimum concentration was determined in the studies presented here as the smallest concentration of base which blocked a nerve exposed to it

6 The length of the anesthetized section of nerve should be greater than 10 mm to prevent the nerve impulse from "jumping over" the anesthetized section To avoid recording an electrotonic potential at the postanesthetic recording site the anesthetized section of nerve was 32 mm (see p 53)

7 Since the amplitude of the action potential increased with increased resistance between the recording electrodes (see p 11) resistance was measured frequently If the resistance between the recording electrodes changed during the experiment, the amplitude was corrected accordingly since it was directly proportional with resistance

The variation in resistance between the recording electrodes, which occurred in these experiments, did not affect the conduction velocity

8 Since the amplitude of the action potential was only slightly dependent on temperature (see p 18) a correction for the small variations in temperature which could occur in these experiments was not considered necessary The conduction velocity was corrected for variations in temperature since a temperature change of 1°C corresponded to a conduction velocity change of 1 m/sec (see p 18) All conduction velocities were corrected to 22°C

9 With moderate stretch of the nerve a change in action potential amplitude occurred on account of the increased resistance between the recording electrodes (see p 46) The degree of stretch was not allowed to exceed 10 per cent and no alterations during the experiment were allowed, unless the resistance was recorded and corrected for The conduction velocity was not dependent on the degree of stretch as long as stretch did not exceed 20 per cent

10 The chamber was bubbled with 97 per cent oxygen and 3 per cent carbon dioxide (see p 31)

11 The chamber was maintained moist to prevent the nerve from drying (see p 23)

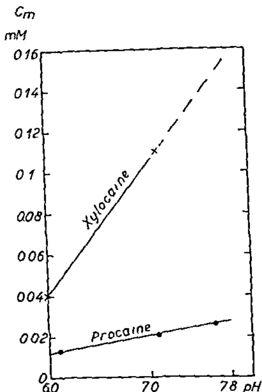


Fig 18 Minimum concentration of procaine and xylocaine base as a function of pH Ordinate minimum base concentration of procaine and xylocaine in mM Abscissa pH

for 6–8 hours (see page 24 and 76) After this time it must be assumed that the outer concentration of anesthetic was equilibrated with the interfibrillar fluid The minimum concentration was determined at various pH values The results are presented in Table 3 and 4 and Figure 18 The minimum concentration of procaine was 4–5 times lower than of xylocaine and the minimum concentration for both local anesthetics diminished with diminishing pH of the solution

The time course of xylocaine and procaine anesthesia.

The effect of xylocaine and procaine on isolated frog nerve was followed using the gradual diminution in amplitude of the nerve action potential as measure of the development of anesthesia

The concentration of xylocaine and procaine hydrochloride varied between 1 and 10 mM The pH values in the experiments varied between 5 67 and 7 8 For the 17 experiments with xylocaine 87 per cent of the pH values lay between 6 75 and 7 55 with a mean value of 7 08 In the case of procaine

Table 3

Minimum concentration of xylocaine base at different pH

xylocaine base concentration (C_B) mM	pH	number of experiments	duration of experiments hours	final action potential in per cent of the amplitude in Ringer's
0 23	7 10	12	2	0
0 15	7 20	10	8	0
0 13	7 17	10	6	0
0 10	7 10	10	7	0 3 8
0 10	6 0	10	6	0
0 06	6 0	10	6	0
0 04	6 0	10	8	4 nerves 0 5 nerves 0 2 2 1 nerve 10

From these experiments the minimum concentration for xylocaine base was estimated to be 0 11 mM at pH 7 10 and 0 04 mM at pH 6 0

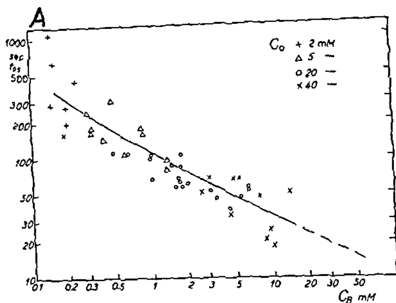
Table 4

Minimum concentration of procaine base at different pH

procaine base concentration (C_B) mM	pH	number of experiments	duration of experiments hours	final action potential in per cent of the amplitude in Ringer's
0 026	7 65	10	7 5	4 nerves 0 4 nerves 3 4 6 9 2 nerves 20–35
0 030	7 18	10	6	0
0 024	7 08	9	7	4 nerves 0 5 nerves 0 3 6 24 71 %
0 014	7 15	10	6 19	3 nerves 13 31 40 7 nerves 0 9 5
0 013	6 12	10	6	6 nerves 0 4 nerves 13 19 81 90

From these experiments the minimum concentration for procaine base was estimated to be

0 026 mM at pH 7 65
0 021 mM at pH 7 08
0 013 mM at pH 6 12



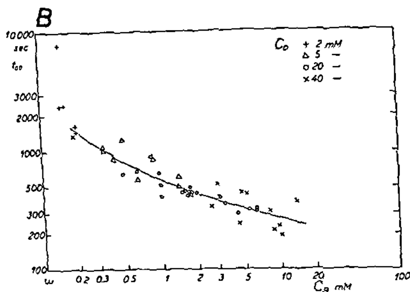


Fig 20 Time to (A) half action potential amplitude ($t_{0.5}$) and (B) to block ($t_{0.01}$) as a function of xylocaine base concentration in experiments with different hydrochloride concentrations (C_o) (temperature range 20–25 ° C, see footnote p 000)

Ordinate seconds after application of the anesthetic (logarithmic scale)

Abscissa xylocaine base concentration (C_B) in mM (logarithmic scale)

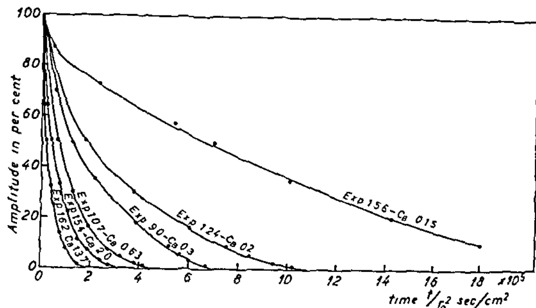


Fig 19 Examples of the time course of the effect of various concentrations of xylocaine base on action potential amplitude The experiments illustrate that anesthesia occurs more rapidly with increasing concentration (temperature range 21–23 9°C*))
Ordinate action potential amplitude as per cent of the amplitude in Ringer s
Abscissa time expressed as t/r_0^2 to compensate for differences in nerve radius (r_0) t is *the time after application of the anesthetic*
The numbers on the curves indicate the number of the experiment and the concentration of xylocaine base (C_B) in mM

the pH range was 6 8–7 8, *but 11 of the 17 experiments were carried out at* pH 7 5 and 7 8 To ensure that the pH of the nerve did not alter in the course of the experiment all nerves were placed for one hour before the experiment in Ringer s solution with the same pH as that of the anesthetic solution to be used The experiments were carried out at 18-26°C

Examples of the effect of various concentrations of xylocaine base are illustrated in Figure 19 The amplitude began to fall immediately when the nerve was placed in the anesthetic solution *The amplitude diminished most per unit time just after the application of xylocaine and the diminution then progressed more slowly to full block* The course of anesthesia was accelerated when the base concentration was increased

The time course of diminution of the action potential amplitude during anesthesia was described by the time at *which the amplitude* reached half its original value ($t_{0.5}$) and blocking time ($t_{0.01}$) which is the time required for the amplitude to fall to 1 per cent of its original value The time to half amplitude of the action potential and to blocking became shorter with *increasing concentration of base* (Figures 20, 21)

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*) As to the influence of difference in temperature cf p 74

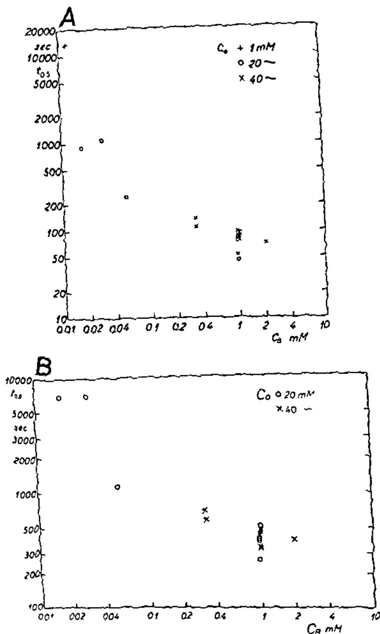


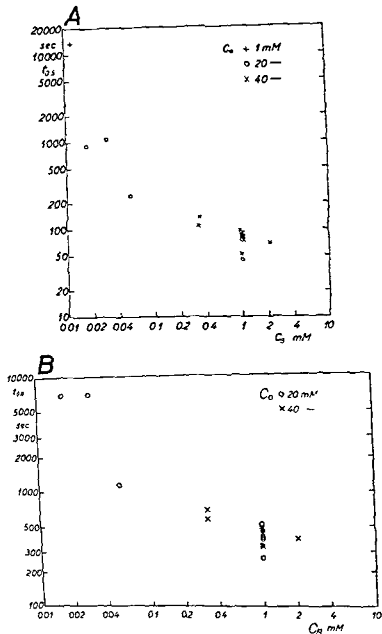
Fig. 21. Time to (A) half action potential amplitude ($t_{0.5}$) and (B) to block ($t_{0.01}$) as a function of procaine base concentration in experiments with different hydrochloride concentrations (C_0) (temperature range 18.6–25.2 C, see footnote p. 62)
Ordinate: seconds after application of the anesthetic (logarithmic scale)
Abscissa: procaine base concentration (C_B) in mM (logarithmic scale)

5

Table 5

Time to half action potential amplitude ($t_{0.5}$) and to block ($t_{0.01}$) in experiments with xylocaine (nerves with sheath)

experiment no	temperature °C	nerve radius (r_0) mm	action potential amplitude in Ringer's mV	hydrochloride concentration (C_0) mM	pH	base concentration (C_B) mM	$t_{0.5}$ sec	$t_{0.5}/r_0^2$ sec/cm²	$t_{0.01}$ sec	$t_{0.01}/r_0^2$ sec/cm²
132	23.1	0.39	7.3	1	6.70	0.05	720	46.1×10^4	—	—
156	22.5	0.39	11.0	2	6.88	0.15	108	69.7×10^4	7800	50.3×10^5
115	23.6	0.41	3.6	2	6.88	0.15	285	16.9×10^4	2440	14.5×10^5
123	21.5	0.40	9.2	2	6.90	0.16	630	39.4×10^4	2490	15.6×10^5
102	22.4	0.41	5.0	40	5.67	0.19	160	9.5×10^4	1340	7.9×10^5
124	23.9	0.40	4.8	2	7.00	0.2	270	16.9×10^4	1633	10.2×10^5
126	28.5	0.38	5.6	2	7.02	0.2	198	13.6×10^4	1452	10.0×10^5
116	21.8	0.38	4.7	2	7.09	0.24	441	30.4×10^4	—	—
90	23.1	0.43	7.4	5	6.77	0.30	210	12.9×10^4	1250	6.7×10^5
92	23.4	0.42	3.6	5	6.84	0.33	165	9.3×10^4	1008	5.7×10^5
136	24.2	0.45	7.2	5	6.84	0.33	178	8.6×10^4	1097	5.3×10^5
91	23.1	0.45	5.7	5	6.92	0.41	148	7.3×10^4	840	4.1×10^5
93	23.4	0.57	5.8	5	6.98	0.48	300	9.1×10^4	1250	3.9×10^5
106	23.4	0.39	4.5	20	6.38	0.48	105	6.9×10^4	635	4.1×10^5
107	22.3	0.40	4.9	20	6.50	0.63	105	6.6×10^4	652	4.1×10^5
86	25.1	0.38	3.9	5	7.11	0.63	110	7.6×10^4	560	3.9×10^5
94	23.3	0.43	6.1	5	7.28	0.85	178	9.5×10^4	807	4.3×10^5
84	23.1	0.43	4.5	5	7.29	0.87	150	8.1×10^4	860	4.6×10^5
99	23.6	0.35	6.4	20	6.70	0.96	90	7.5×10^4	635	5.3×10^5
157	22.3	0.40	5.1	20	6.70	1.0	85	5.3×10^4	507	3.1×10^5
158	24.0	0.41	6.4	20	6.70	1.0	60	3.6×10^4	402	2.4×10^5
87	25.2	0.38	2.5	5	7.54	1.4	80	5.5×10^4	495	3.4×10^5
88	25.2	0.43	4.5	5	7.55	1.4	96	5.2×10^4	590	3.2×10^5
100	24.2	0.37	6.1	20	6.89	1.5	76	5.5×10^4	440	3.2×10^5
101	23.6	0.38	4.8	20	6.92	1.59	57	4.0×10^4	458	3.3×10^5
76	24.0	0.41	6.6	20	6.93	1.62	67	3.4×10^4	414	2.4×10^5
95	22.6	0.43	1.8	20	6.95	1.74	105	5.8×10^4	480	2.7×10^5
96	21.9	0.38	4.4	20	6.97	1.78	82	5.8×10^4	450	3.1×10^5
103	21.5	0.36	4.6	20	7.01	1.8	57	4.4×10^4	442	3.4×10^5
128	22.3	0.42	5.5	20	6.99	1.8	46	2.6×10^4	444	2.5×10^5
154	22.7	0.40	9.5	20	7.02	2.0	60	3.7×10^4	440	2.7×10^5
109	20.0	0.39	5.4	40	6.83	2.63	51	3.3×10^4	337	2.2×10^5
159	21.3	0.44	9.6	40	6.90	3.0	67	3.5×10^4	510	2.6×10^5
104	21.6	0.38	7.7	20	7.23	3.1	53	3.7×10^4	405	2.8×10^5
79	22.8	0.39	7.8	20	7.30	3.47	45	2.9×10^4	355	2.3×10^5
108	22.8	0.40	4.2	20	7.41	4.36	37	2.3×10^4	292	1.8×10^5
130	24.1	0.37	5.3	40	7.00	4.47	31	2.3×10^4	241	1.8×10^5
110	21.8	0.49	4.5	40	7.12	4.78	65	2.7×10^4	442	1.8×10^5
111	21.3	0.44	4.7	40	7.16	5.13	66	3.4×10^4	429	2.2×10^5
161	22.2	0.46	6.3	20	7.57	5.5	46	2.2×10^4	330	1.6×10^5
98	23.7	0.42	6.9	20	7.66	6.3	52	2.9×10^4	310	1.7×10^5
97	22.7	0.42	4.8	20	7.64	6.31	56	3.2×10^4	312	1.8×10^5
160	21.2	0.44	7.5	40	7.40	8.1	47	2.4×10^4	306	1.6×10^5
112	22.0	0.38	3.1	40	7.44	8.71	21	1.5×10^4	210	1.5×10^5
114	23.1	0.42	4.3	40	7.49	9.55	24	1.4×10^4	235	$1.3 \; 10^5$
113	22.3	0.38	3.7	40	7.52	10.0	18	1.2×10^4	192	1.3×10^5
162	21.0	0.43	8.2	40	7.72	13.7	50	2.7×10^4	369	2.0×10^5



ig 21 Time to (A) half action potential amplitude ($t_{0.5}$) and (B) to block ($t_{0.01}$) as a ction of procaine base concentration in experiments with different hydrochloride con trations (C_0) (temperature range 18 6–25 2° C, see footnote p 62)
linate seconds after application of the anesthetic (logarithmic scale)
scissa procaine base concentration (C_B) in mM (logarithmic scale)

5

Table 5

Time to half action potential amplitude ($t_{0.5}$) and to block ($t_{0.01}$) in experiments with xylocaine (nerves with sheath)

experiment no	temperature °C	nerve radius (r_0) mm	action potential amplitude in Ringer's mV	hydrochloride concentration (C_0) mM	pH	base concentration (C_B) mM	$t_{0.5}$ sec	$\frac{t_{0.5}}{r_0^2}$ sec/cm²	$t_{0.01}$ sec	$\frac{t_{0.01}}{r_0^2}$ sec/cm²
132	23.1	0.39	7.3	1	6.70	0.05	720	46.1×10^4	—	—
156	22.5	0.39	11.0	2	6.88	0.15	108	69.7×10^4	7800	50.3×10^5
115	23.6	0.41	3.6	2	6.88	0.15	285	16.9×10^4	2440	14.5×10^5
123	21.5	0.40	9.2	2	6.90	0.16	630	39.4×10^4	2190	15.6×10^5
102	22.4	0.41	5.0	40	5.67	0.19	160	9.5×10^4	1340	7.9×10^5
124	23.9	0.40	4.8	2	7.00	0.2	270	16.9×10^4	1633	10.2×10^5
126	28.5	0.38	5.6	2	7.02	0.2	198	13.6×10^4	1452	10.0×10^5
116	21.8	0.38	4.7	2	7.09	0.24	411	30.4×10^4	—	—
90	23.1	0.43	7.4	5	6.77	0.30	210	12.9×10^4	1250	6.7×10^5
92	23.4	0.42	3.6	5	6.81	0.33	165	9.3×10^4	1008	5.7×10^5
136	24.2	0.45	7.2	5	6.84	0.33	178	8.6×10^4	1097	5.3×10^5
91	23.1	0.45	5.7	5	6.92	0.41	148	7.3×10^4	840	4.1×10^5
93	23.4	0.57	5.8	5	6.98	0.48	300	9.4×10^4	1250	3.9×10^5
106	23.4	0.39	4.5	20	6.38	0.48	105	6.9×10^4	635	4.4×10^5
107	22.3	0.40	4.9	20	6.50	0.63	105	6.6×10^4	652	4.1×10^5
86	25.1	0.38	3.9	5	7.11	0.63	110	7.6×10^4	560	3.9×10^5
94	23.3	0.43	6.1	5	7.28	0.85	178	9.5×10^4	807	4.3×10^5
84	23.1	0.43	4.5	5	7.29	0.87	150	8.1×10^4	800	4.6×10^5
99	23.6	0.35	6.4	20	6.70	0.96	90	7.5×10^4	635	5.3×10^5
157	22.3	0.40	5.1	20	6.70	1.0	85	5.3×10^4	507	3.1×10^5
158	21.0	0.41	6.4	20	6.70	1.0	60	3.6×10^4	402	2.4×10^5
87	25.2	0.38	2.5	5	7.54	1.1	80	5.5×10^4	495	3.4×10^5
88	25.2	0.43	4.5	5	7.55	1.1	96	5.2×10^4	590	3.2×10^5
100	21.2	0.37	6.1	20	6.89	1.5	76	5.5×10^4	440	3.2×10^5
101	23.6	0.38	4.8	20	6.92	1.59	57	4.0×10^4	458	3.3×10^5
76	24.0	0.44	6.6	20	6.93	1.62	67	3.4×10^4	414	2.1×10^5
95	22.6	0.43	1.8	20	6.95	1.74	105	5.8×10^4	480	2.7×10^5
96	21.9	0.38	4.4	20	6.97	1.78	82	5.8×10^4	450	3.1×10^5
103	21.5	0.36	4.6	20	7.01	1.8	57	4.4×10^4	412	3.4×10^5
128	22.3	0.42	5.5	20	6.99	1.8	46	2.6×10^4	444	2.5×10^5
151	22.7	0.40	9.5	20	7.02	2.0	60	3.7×10^4	440	2.7×10^5
109	20.0	0.39	5.4	10	6.83	2.63	51	3.3×10^4	337	2.2×10^5
159	21.3	0.44	9.6	40	6.90	3.0	67	3.5×10^4	510	2.6×10^5
104	21.6	0.38	7.7	20	7.23	3.1	53	3.7×10^4	405	2.8×10^5
79	22.8	0.39	7.8	20	7.30	3.17	45	2.9×10^4	355	2.3×10^5
108	22.8	0.40	4.2	20	7.44	4.36	37	2.3×10^4	292	1.8×10^5
130	24.1	0.37	5.3	10	7.09	4.17	31	2.3×10^4	244	1.8×10^5
110	21.8	0.49	4.5	10	7.12	4.78	65	2.7×10^4	112	1.8×10^5
111	21.3	0.44	4.7	10	7.10	5.13	46	3.4×10^4	424	2.2×10^5
161	22.2	0.46	6.3	20	7.57	5.5	46	2.2×10^4	330	1.6×10^5
98	23.7	0.42	6.9	20	7.66	6.3	52	2.9×10^4	310	1.7×10^5
97	22.7	0.42	4.8	20	7.64	6.31	56	3.2×10^4	312	1.8×10^5
160	21.2	0.44	7.5	40	7.40	8.1	17	2.4×10^4	300	1.1×10^5
112	22.0	0.38	3.1	10	7.44	8.71	21	1.5×10^4	216	1.5×10^5
114	23.1	0.42	4.3	10	7.49	9.55	21	1.1×10^4	235	1.3×10^5
113	22.3	0.38	3.7	10	7.52	10.0	18	1.2×10^4	192	1.3×10^5
162	21.0	0.43	8.2	10	7.72	13.7	50	2.7×10^4	369	2.0×10^5

Table 7

Standard deviation (S D) and mean error (ε) of the time to half amplitude and to block in per cent of the mean value in different base concentration ranges (for xylocaine cf Figs 20 and 22, for procaine cf Table 6)

	base concentration range mM	number of experiments	$t_{0.5}$		$t_{0.01}$	
			S D	$\pm\varepsilon$	S D	$\pm\varepsilon$
xylocaine	0 2–0 90 1 0–4 0 4 0–13 7	14 13 16 12	37 24 40	10 6 10	17 13 29	5 3 6
procaine	1 0	10	21	7	19	6

	C_B/C_m range	number of experiments	$t_{0.5}/r_0^2$		$t_{0.01}/r_0^2$	
			S D	$\pm\varepsilon$	S D	$\pm\varepsilon$
xylocaine	3 4 12*) 15–90	15 24	20 26	5 5	19 17	5 3 5
procaine	37–40	10	14	4	17	6

*) Experiment no 102 (with extremely low pH) at $C_B/C_m = 10.5$ is omitted

at different pH shown in Figure 18 The spread of the corrected values (Table 7) did not differ significantly from that of the uncorrected values. The relatively large spread is probably due to biological variations from frog to frog It may be mentioned that small variations in base concentration in the range near the minimum concentration result in large changes in the time course of anesthesia Such variations in base concentration can occur even with slight alterations in the pH of the solution For example on increase in pH of 0 1 unit can increase the base concentration from 0 15 to 0 19 mM and thereby increase the time to half amplitude ($t_{0.5}$) by a factor of 2 (Figure 20 A) With higher base concentrations some of the variability can perhaps be ascribed to uncertainties in the determination of the moment of application of the local anesthetic in that the action potential amplitude diminishes rapidly after its application

In the case of procaine the number of experiments allowed evaluation of the spread solely at the concentration 1 0 mM procaine base The standard deviation of the individual values and the mean error of $t_{0.5}$, $t_{0.01}$ and $t_{0.5}/r_0^2$, $t_{0.01}/r_0^2$ are shown in Table 7 It is seen that the spread was less when differences in the radius of the nerve were corrected for

Table 6

Time to half action potential amplitude ($t_{0\,5}$) and to block ($t_{0\,01}$) in experiments with procaine (nerves with sheath)

experiment no	temperature °C	nerve radius (r_0) mm	action potential amplitude in Ringer's mV	hydrochloride concentration (C_0) mM	pH	base concentration (C_B) mM	$t_{0\,5}$ sec	$\frac{t_{0\,5}}{r_0^2}$ sec/cm²	$t_{0\,01}$ sec	$\frac{t_{0\,01}}{r_0^2}$ sec/cm²
170	24 0	0 41	4 2	1	7 18	0 012	13800	$8\,2 \times 10^6$	—	—
139	22 2	0 38	6 4	20	6 00	0 017	900	$6\,1 \times 10^5$	8500	$5\,9 \times 10^6$
151	22 3	0 45	5 6	20	6 20	0 030	1000	$4\,9 \times 10^5$	7000	$3\,5 \times 10^6$
138	22 8	0 47	6 1	20	6 50	0 054	240	$1\,1 \times 10^5$	1127	$5\,2 \times 10^5$
153	22 3	0 43	7 2	40	7 00	0 32	135	$7\,5 \times 10^4$	666	$3\,7 \times 10^5$
135	24 5	0 45	7 4	40	7 00	0 33	97	$4\,8 \times 10^4$	568	$2\,9 \times 10^5$
140	25 0	0 35	4 8	20	7 80	1 0	45	$3\,7 \times 10^4$	261	$2\,2 \times 10^5$
141	21 5	0 36	7 9	20	7 80	1 0	75	$5\,8 \times 10^4$	450	$3\,5 \times 10^5$
145	18 6	0 38	8 0	20	7 80	1 0	83	$5\,9 \times 10^4$	385	$2\,7 \times 10^5$
149	23 7	0 41	8 8	20	7 80	1 0	82	$4\,9 \times 10^4$	462	$2\,8 \times 10^5$
150	23 9	0 45	7 1	20	7 80	1 0	81	$3\,9 \times 10^4$	393	$1\,9 \times 10^5$
142	22 0	0 36	6 9	40	7 50	1 0	55	$4\,2 \times 10^4$	310	$2\,6 \times 10^5$
143	21 9	0 38	5 8	40	7 50	1 0	81	$5\,7 \times 10^4$	475	$3\,3 \times 10^5$
147	25 7	0 40	5 3	40	7 55	1 1	85	$5\,3 \times 10^4$	438	$2\,7 \times 10^5$
148	26 0	0 38	—	40	7 50	1 0	68	$4\,7 \times 10^4$	321	$2\,3 \times 10^5$
146	25 3	0 43	6 2	20	7 80	1 0	90	$5\,0 \times 10^4$	510	$2\,8 \times 10^5$
137	23 2	0 39	6 5	40	7 80	2 0	70	$4\,5 \times 10^4$	390	$2\,6 \times 10^5$

Variability of the experimental results

Xylocaine In Figure 20 the curves are drawn around which the experimental values for the time to half and to 1 per cent of the initial action potential amplitude are grouped In 3 ranges of base concentration the spread of the individual values around the mean was calculated The standard deviation and the mean error are listed in Table 7 The variability was least when the base concentration was in the range 1 0 1 0 mM and the blocking time showed less spread than the time to half amplitude of the action potential In Figure 20 no correction has been made for the variations in pH and the resultant variations in minimum concentration Furthermore no correction has been made for differences in the nerve diameter which can give rise to variations, in that BENNETT et al (1942) and EHRENBERG (1948) found that the blocking time was proportional to the square of the nerve radius To eliminate the effect of these factors on the variability Figures 22 & 23 show the corrected time (t/r_0^2, when r_0 is the radius of the nerve) as a function of the ratio between base and minimum concentration, making use of the minimum concentrations

Table 8

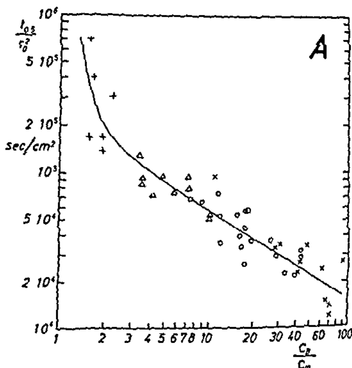
Time to half action potential amplitude ($t_{0.5}$) and to block ($t_{0.01}$) at different xylocaine base concentrations (C_B/C_m) as a function of the xylocaine acid concentration

experiment no	hydrochloride concentration (C_s) mM	pH	acid concentration (C_{BH^+}) mM	base concentration (C_B) mM	minimum concentration *) (C_m) mM	C_B/C_m	$\frac{t_{0.5}}{r_0^2}$ sec/cm²	$\frac{t_{0.01}}{r_0^2}$ sec/cm²
84	5	7 29	4 13	0 87	0 12	7 2	8 1 × 10⁴	4 6 × 10⁵
94	5	7 28	4 15	0 85	0 12	7 0	9 5 × 10⁴	4.3 × 10⁵
10[illegible]	20	6 38	19 52	0 48	0 07	7 4	6 9 × 10⁴	4 1 × 10⁵
104	20	7 23	16 9	3 1	0 12	25 8	3 7 × 10⁴	2 8 × 10⁵
9	20	7 30	16 53	3 47	0 12	28 2	2 9 × 10⁴	2 3 × 10⁵
10[illegible]	40	6 83	37 37	2 63	0 09	28 2	3 3 × 10⁴	2 2 × 10⁵
159	40	6 90	37 0	3 0	0 10	30 0	3 5 × 10⁴	2 6 × 10⁵
161	20	7 57	14 5	5 5	0 14	39 3	2 2 × 10⁴	1 6 × 10⁵
98	20	7 66	13 7	6.3	0 15	42 8	2 9 × 10⁴	1 7 × 10⁵
97	20	7 64	13 69	6 31	0 15	43 2	3 2 × 10⁴	1 8 × 10⁵
130	40	7 09	35 53	4 47	0 11	40 6	2 3 × 10⁴	1 8 × 10⁵
110	40	7 12	35 22	4 78	0 11	42 7	2 7 × 10⁴	1 8 × 10⁵
111	40	[illegible] 16	34 87	5 13	0 11	46 7	3 4 × 10⁴	2.2 × 10⁵

*) Values from Fig 18

Base concentration of the anesthetic versus the acid concentration as anesthetic agent

Assuming that it is solely the base which is the active anesthetic factor, the time course of anesthesia would be determined by the ratio C_B/C_m To test this assumption the time course of anesthesia can be compared in experiments where C_B C_m was constant but where the concentration of xylocaine acid differed The result of such a comparison is given in Table 8 In the case of xylocaine the base concentration was 7 28 and 42 times the minimum concentration and at each of these levels the acid concentration was varied 2 5 times An increase in acid concentration by up to 4 7 times did not affect



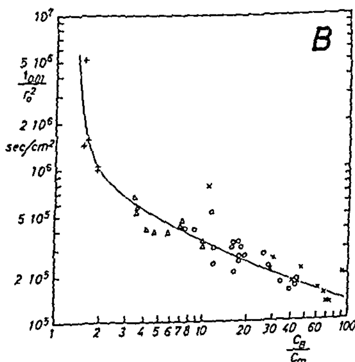


Fig. 22. The time to (A) half action potential amplitude and (B) to block for different concentrations of xylocaine base, corrected for differences in nerve radius and pH (temperature range 20–25.5°C, see footnote p. 62).

Ordinate: t/r_0^2 where t is the time in seconds after application of the anesthetic and r_0 the nerve radius in centimeters (logarithmic scale).

Abscissa: xylocaine base concentration (C_B) in units of the minimum concentration (C_m) (logarithmic scale).

The minimum concentration used is that found at the pH of the individual experiments. The symbols indicate hydrochloride concentrations: + = 2 mM, – 5 mM, ○ = 20 mM, × = 40 mM.

Table 8.

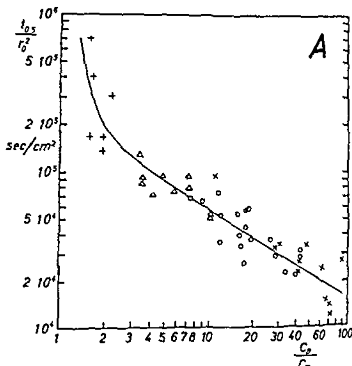
Time to half action potential amplitude ($t_{0.5}$) and to block ($t_{0.01}$) at different xylocaine base concentrations (C_B/C_m) as a function of the xylocaine acid concentration

experiment no	hydrochloride concentration (C_0) mM	pH	acid concentration (C_{BH^+}) mM	base concentration (C_B) mM	minimum concentration *) (C_m) mM	C_B/C_m	$\frac{t_{0.5}}{r_0^2}$ sec/cm²	$\frac{t_{0.01}}{r_0^2}$ sec/cm²
84	5	7.29	4.13	0.87	0.12	7.2	8.1×10^4	4.6×10^5
94	5	7.28	4.15	0.85	0.12	7.0	9.5×10^4	4.3×10^5
106	20	6.38	19.52	0.48	0.07	7.1	6.9×10^4	4.1×10^5
104	20	7.23	16.9	3.1	0.12	25.8	3.7×10^4	2.8×10^5
79	20	7.30	16.53	3.47	0.12	28.2	2.9×10^4	2.3×10^5
109	40	6.83	37.37	2.63	0.09	28.2	3.3×10^4	2.2×10^5
159	40	6.90	37.0	3.0	0.10	30.0	3.5×10^4	2.6×10^5
161	20	7.57	14.5	5.5	0.14	39.3	2.2×10^4	1.6×10^5
98	20	7.66	13.7	6.3	0.15	42.8	2.9×10^4	1.7×10^5
97	20	7.64	13.69	6.31	0.15	43.2	3.2×10^4	1.8×10^5
130	40	7.09	35.53	4.47	0.11	40.6	2.3×10^4	1.8×10^5
110	40	7.12	35.22	4.78	0.11	42.7	2.7×10^4	1.8×10^5
111	40	7.16	34.87	5.13	0.11	46.7	3.4×10^4	2.2×10^5

*) Values from Fig. 18

Base concentration of the anesthetic versus the acid concentration as anesthetic agent.

Assuming that it is solely the base which is the active anesthetic factor, the time course of anesthesia would be determined by the ratio C_B/C_m. To test this assumption the time course of anesthesia can be compared in experiments where C_B/C_m was constant but where the concentration of xylocaine acid differed. The result of such a comparison is given in Table 8. In the case of xylocaine the base concentration was 7, 28 and 42 times the minimum concentration and at each of these levels the acid concentration was varied 2.5 times. An increase in acid concentration by up to 4.7 times did not affect



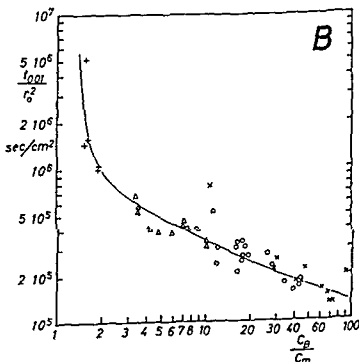


Fig. 22. The time to (A) half action potential amplitude and (B) to block for different concentrations of xylocaine base, corrected for differences in nerve radius and pH (temperature range 20–25.5°C; see footnote p. 62).

Ordinate: t/r_0^2 where t is the time in seconds after application of the anesthetic and r_0 the nerve radius in centimeters (logarithmic scale).

Abscissa: xylocaine base concentration (C_B) in units of the minimum concentration (C_m) (logarithmic scale).

The minimum concentration used is that found at the pH of the individual experiments. The symbols indicate hydrochloride concentrations: + = 2 mM; △ = 5 mM; ○ = 20 mM; × = 40 mM.

Table 8.

Time to half action potential amplitude ($t_{0.5}$) and to block ($t_{0.01}$) at different xylocaine base concentrations (C_B/C_m) as a function of the xylocaine acid concentration

experiment no	hydrochloride concentration (C_a) mM	pH	acid concentration (C_{BH^+}) mM	base concentration (C_B) mM	minimum concentration *) (C_m) mM	C_B/C_m	$\frac{t_{0.5}}{r_0^2}$ sec/cm²	$\frac{t_{0.01}}{r_0^2}$ sec/cm²
84	5	7.29	4.13	0.87	0.12	7.2	8.1×10^4	4.6×10^5
94	5	7.28	4.15	0.85	0.12	7.0	9.5×10^4	4.3×10^5
106	20	6.38	19.52	0.48	0.07	7.1	6.9×10^4	4.1×10^5
104	20	7.23	16.9	3.1	0.12	25.8	3.7×10^4	2.8×10^5
79	20	7.30	16.53	3.47	0.12	28.2	2.9×10^4	2.3×10^5
109	40	6.83	37.37	2.63	0.09	28.2	3.3×10^4	2.2×10^5
159	40	6.90	37.0	3.0	0.10	30.0	3.5×10^4	2.6×10^5
161	20	7.57	14.5	5.5	0.14	39.3	2.2×10^4	1.6×10^5
98	20	7.66	13.7	6.3	0.15	42.8	2.9×10^4	1.7×10^5
97	20	7.64	13.69	6.31	0.15	43.2	3.2×10^4	1.8×10^5
130	40	7.09	35.53	4.47	0.11	40.6	2.3×10^4	1.8×10^5
110	40	7.12	35.22	4.78	0.11	42.7	2.7×10^4	1.8×10^5
111	40	7.16	34.87	5.13	0.11	46.7	3.4×10^4	2.2×10^5

*) Values from Fig. 18

Base concentration of the anesthetic versus the acid concentration as anesthetic agent.

Assuming that it is solely the base which is the active anesthetic factor, the time course of anesthesia would be determined by the ratio C_B/C_m. To test this assumption the time course of anesthesia can be compared in experiments where C_B/C_m was constant but where the concentration of xylocaine acid differed. The result of such a comparison is given in Table 8. In the case of xylocaine the base concentration was 7, 28 and 42 times the minimum concentration and at each of these levels the acid concentration was varied 2.5 times. An increase in acid concentration by up to 4.7 times did not affect

the time course of anesthesia as long as C_B/C_m was constant On the other hand an increase of C_B/C_m by 4 times with unaltered acid concentration reduced the half and blocking time by more than 10 per cent Hence, in the experiments reported here the course of anesthesia was determined primarily by the concentration of base

In the case of procaine, too, the course of anesthesia was a function of the base concentration (Fig 21) though the findings were less clear cut than with xylocaine, since in the main the procaine experiments were carried out at base concentrations such that slight changes could influence the time course only slightly

The time course of xylocaine anesthesia in nerves without sheath.

The nerve sheath was removed over that portion of the nerve to be exposed to the anesthetic (p 16) and the reduction in action potential amplitude after exposure to various concentrations of xylocaine base was followed

The time course of anesthesia in nerves without sheath was qualitatively the same as in nerves with sheath Immediately after application of the anesthetic the amplitude fell rapidly, and this reduction in amplitude continued at a slower rate to block The results of 17 experiments on nerves without sheath are listed in Table 9

With increasing base concentration the amplitude of the action potential diminished more rapidly for concentrations between 0 1 and 1 6 mM xylocaine base In the concentration range 1 6–15 5 mM the time to half amplitude and the blocking time were largely unaltered

The spread of the experimental results was calculated from 8 experiments in which the xylocaine base concentration was 1 6 2 3 mM The standard deviation for half-time and blocking time was 26 per cent and 29 per cent and the mean error was ± 9 per cent and ± 10 per cent The standard deviations for $t_{0.5}/r_0^2$ and $t_{0.01}/r_0^2$ were 40 and 35 per cent respectively, i e greater than the values uncorrected for the nerve thickness The explanation may be that the diameter of sheathless nerves increased when they were placed in Ringer s solution (TRUANT and LANZONI 1952 FRANKENHAEUSER and NYSTROM 1951)

Comparison of the time course of anesthesia in nerves with and without sheath

No systematic experiments were carried out on the same nerve with and without sheath since preliminary experiments had demonstrated that it was impossible to wash the nerve completely free of anesthetic substance after the first anesthesia

The importance of the nerve sheath for the time course of anesthesia is

Table 9

Time to half action potential amplitude ($t_{0.5}$) and to block ($t_{0.01}$) in experiments with xylocaine (nerves without sheath)

experiment no	temperature °C	nerve radius (r_0) mm	action potential amplitude in Ringer's mV	hydrochloride concentration (C_0) mM	pH	base concentration (C_B) mM	$t_{0.5}$ sec	$\frac{t_{0.5}}{r_0^2}$ sec/cm² × 10⁴	$t_{0.01}$ sec	$\frac{t_{0.01}}{r_0^2}$ sec/cm² × 10⁵
133	22.0	0.33	0.6	1	7.00	0.1	370	34.0	—	—
125	29.4	0.33	2.2	2	7.00	0.2	99	9.1	885	8.1
189	21.8	0.37	1.5	5	6.75	0.3	140	10.2	550	4.0
127	22.7	0.39	4.1	20	6.92	1.6	17	1.1	172	1.1
193	25.3	0.31	2.4	20	6.97	1.8	6	0.7	53	0.6
194	21.9	0.32	1.8	20	6.97	1.8	20	2.0	117	1.1
196	23.6	0.36	3.1	20	7.00	1.9	17	1.3	148	1.1
192	24.8	0.31	3.7	20	7.00	1.9	17	1.8	133	1.4
168	24.3	0.37	2.7	20	7.02	2.0	14	1.0	110	0.8
177	25.1	0.45	4.5	20	7.10	2.3	14	0.7	83	0.4
173	25.1	0.46	3.1	20	7.10	2.3	14	0.7	119	0.6
175	23.9	0.40	2.0	40	7.16	5.0	12	0.8	90	0.6
166	23.1	0.40	2.7	40	7.22	5.8	31	1.9	202	1.3
169	25.7	0.39	4.1	40	7.40	8.1	17	1.1	162	1.1
176	24.6	0.42	3.8	20	7.85	8.5	21	1.2	168	1.0
198	24.1	0.34	2.8	40	7.50	9.3	12	1.0	120	1.0
167	23.4	0.40	3.5	40	7.80	15.5	24	1.5	159	1.0

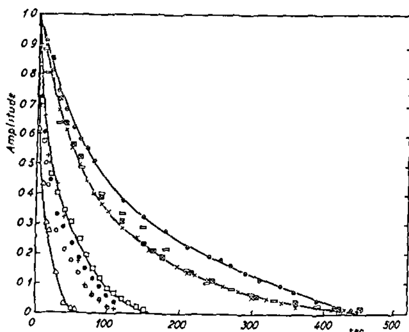


Fig 23 Reduction in action potential amplitude during xylocaine anesthesia in nerves with and without sheath (temperature range 21 5 25 3°C, see footnote p 62) Base concentrations 1 8–2 mM in 5 nerves without (lower curves) and 4 nerves with sheath (upper curves)
Ordinate amplitude in units of the amplitude in Ringer s
Abscissa seconds after application of the anesthetic

Nerves without sheath

- □ Experiment 196 C_B = 1 9 mM, r_0 0 36 mm, pH 7 0
- △ Experiment 193 C_B = 1 8 mM, r_0 0 31 mm, pH 6 97
- ● Experiment 192 C_B = 1 9 mM, r_0 0 31 mm pH 7 0
- ○ Experiment 168 C_B = 2 0 mM, r_0 0 37 mm pH 7 02
- – Experiment 191 C_B = 1 8 mM, r_0 0 32 mm, pH 6 97

Nerves with sheath

- ▭ Experiment 154 C_B = 2 0 mM, r_0 0 40 mm, pH 7 02
- ⊖ *Experiment 96 C_B = 1 8 mM, r_0 0 38 mm, pH 6 97*
- × Experiment 103 C_B = 1 8 mM r_0 0 36 mm pH 7 01
- ⊠ Experiment 128 C_B – 1 8 mM, r_0 0 42 mm, pH 6 99

Table 10.

Time to half action potential amplitude ($t_{0.5}$), to 20 per cent amplitude ($t_{0.2}$) and to block ($t_{0.01}$) in nerves with and without sheath (xylocaine)

	experiment no	base concentration (C_B) mM	nerve radius (r_0) mm	$t_{0.5}$ sec	ratio	$t_{0.2}$ sec	ratio	$t_{0.01}$ sec	ratio
+ sheath	132	0 05	0 39	720	2 0				
— sheath	133	0 1	0 33	370					
+ sheath	126	0 2	0 38	198	2 0	670	1 9	1452	1 6
— sheath	125	0 2	0 33	99		360		885	
+ sheath	90	0 3	0 43	240	1 7	660	2 1	1250	2 3
— sheath	189	0 3	0 37	140		310		550	
+ sheath	76	1 6	0 41	67	3 9	186	3 1	414	2 6
— sheath	127	1 6	0 39	17		60		172	
+ sheath	161	5 5	0 46	44	1 4	124	1.5	330	1 6
— sheath	166	5 8	0 40	31		85		202	
+ sheath	160	8 1	0 44	45	2 6	126	2 3	306	1 9
— sheath	169	8 1	0 39	17		55		162	
+ sheath	114	9 6	0 42	24	2 0	83	2 1	235	2 0
— sheath	198	9 3	0 31	12		39		120	
+ sheath	162	13 7	0 43	50	2 1	143	2 2	369	2 3
— sheath	167	15 5	0 40	24		65		159	
				mean value	2 2		2 2		2 1

illustrated in Figure 23 which shows examples of the diminution in action potential amplitude in the experiments on nerves subjected to xylocaine base concentrations of 1 8–2 0 mM, 5 without sheath and 4 with sheath There was a clear difference in the time course between the two groups Comparing the times when the amplitude of the action potential was reduced to 50 per cent, 20 per cent and 1 per cent, nerves without sheath were anesthetized about 4 times more rapidly than nerves with sheath In this comparison no correction was made for differences in nerve diameter Comparing the time course of the anesthesia in nerves which had the same diameter before removal of the sheath a somewhat smaller difference was found (Table 10) The sheathless nerves were anesthetized about twice as rapidly as matched nerves with sheath

There is no apparent explanation for the finding in these experiments of

a factor 2 between anesthetic times for nerves with and without sheath as compared with a factor 4 in nerves unmatched for diameters and at the same anesthetic concentration. It is possible that in some of the nerves in the "unmatched" experiments the removal of sheath caused a certain separation of the single fibers so that the anesthetic diffused between them more rapidly, although there was no apparent difference in technique.

Table 11.

Time to half action potential amplitude ($t_{0.5}$) and to block ($t_{0.01}$) during xylocaine anesthesia at different temperatures

experiment no	temperature °C	hydrochloride concentration (C_0) mM	pH	*base concentration* (C_B) mM	nerve radius (r_0) mm	$\frac{t_{0.5}}{r_0^2}$ sec/cm²	$\frac{t_{0.01}}{r_0^2}$ sec/cm²
118	3	20	7.20	2.9	0.38	1.0×10^5	6.1×10^5
119	4.5	20	7.18	2.8	0.36	6.1×10^4	4.1×10^5
120	7	20	7.25	3.2	0.35	6.4×10^4	4.0×10^5
127	9	20	7.09	2.3	0.37	4.3×10^4	3.3×10^5
121	15	20	7.08	2.3	0.35	5.1×10^4	3.2×10^5
109	20	40	6.83	2.6	0.39	3.3×10^4	2.2×10^5
159	21.3	10	6.90	3.0	0.41	3.5×10^4	2.6×10^5
104	21.6	20	7.23	3.1	0.38	3.7×10^4	2.8×10^5

The effect of temperature on the course of anesthesia.

In the concentration range 2.3–3.2 mM xylocaine base the time course of anesthesia in nerves with sheath was investigated at temperatures between 3 and 21°C (Table 11, Figure 21). Between 3 and 13°C the time to half amplitude and the time to 1 per cent amplitude diminished 50 per cent ($Q_{10} = 2$). Although each nerve was investigated at one temperature only, the acceleration of the anesthesia could not be explained by individual variations or by differences in base concentration. In the temperature range 13–21°C there was no significant change in the time course of the anesthesia. In some experiments the temperature was above 22°C (maximally 26°C *)). These were performed with base concentrations below 2.3 mM or above 3.2 mM, and the influence of temperature could therefore not be evaluated.

*) In one exceptional experiment – no. 126 – the temperature was 28.5°C.

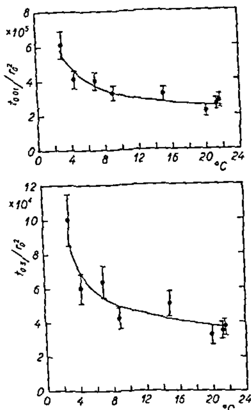


Fig 24 Time to half the action potential amplitude ($t_{0\,5}$) and to block ($t_{0\,01}$) in xylocaine anesthesia as a function of temperature Base concentration 2.3 3 2 mM
Ordinate t/r_0^2 where t is the time in seconds after application of the anesthetic and r_0 the nerve radius (sec/cm²)
Abscissa temperature in centigrades
The experiments were carried out with different nerves at the different temperatures The standard deviation is indicated by the vertical lines

directly However if $t_{0\,5}$ and $t_{0\,01}$ were corrected to a concentration range of 2 3 mM (Fig 22) also in the experiments performed at 22–26°C the influence of differences in temperature was negligeable These findings justify to pool all experiments performed at room temperature, within the temperature range 18 26°C

DISCUSSION

Minimum concentration.

The minimum concentration of procaine was determined for the first time by GROS (1910) on the basis of clinical experiments carried out by HEINEKE and LAWEN (1905). TREVAN and BOOCK (1927) used local anesthetics applied to the cornea of rabbits with the corneal reflex as indicator of anesthetic effect. BOEHMINGHAUS and KOCHMANN (1929) determined the minimum concentration for procaine using a nerve-muscle preparation. To ensure that there was equilibrium between the anesthetic in and outside the nerve BOEHMINGHAUS and KOCHMANN insist that the bathing time should be extended to 12–24 hours when low concentrations are used. It appears, however, from BOEHMINGHAUS and KOCHMANN's own control experiments with a nerve in Ringer's for 24 hours, that such a long experimental time results in an increase in the threshold of the nerve to stimulation. With a higher threshold to stimulation it might be expected that the minimum concentration of the anesthetic would decrease. Therefore the experimental time should not be longer than necessary. In the experiments reported here the minimum concentration was determined which caused the action potential to disappear after 6–8 hours of exposure to the anesthetic. That this exposure time was considered sufficient to ensure equilibrium between the inner and outer solutions is based on the following considerations.

1) The minimum concentration found in experiments using 6–8 hours exposure time was lower or equal to that of other investigators both for xylocaine and procaine (see Table 12).

2) In the experiments concerned with the time course of anesthesia the lowest xylocaine base concentration able to block a nerve was 0.15 mM. Extrapolating the time required to reduce the action potential to one per cent of its original value to lower base concentration a minimum concentration was found of the same order as that determined directly after 6–8 hours (0.1 mM xylocaine base). At the minimum concentration $t_{0.01}$ is infinite, i.e. $1/t_{0.01} = 0$. The minimum concentration was therefore determined by extrapolating to the concentration at which $1/t_{0.01} = 0$.

The experiments concerned with the determination of the minimum concentration were carried out on nerves which were 25 per cent thinner than the nerves used in the investigation of the whole course of anesthesia. Therefore, the blocking time in these experiments was about 60 per cent of that in the experiments on the time course (maximally about $2\frac{1}{2}$ hours). Thus, using an experimental exposure time of 6–8 hours there should be a reasonable safety margin that equilibrium be reached between the inner and

Table 12

Minimum concentrations for procaine and xylocaine determined by various authors

author	year	anesthetic	method	type of nerve	pH	minimum concentration	
						hydrochloride	free base mM
Heineke & Laewen	1905	procaine	infiltration	sensitive human	*	0.1%	
Trevan & Boock	1927	procaine	cornea	sensitive rabbit	*		0.13
Boeminghaus & Kochmann	1929	procaine	nerve muscle preparation***)	sensitive	7.0?	0.05%	0.15*)
				motor *Rana temporaria*	7.0?	0.075%	0.23*)
Bennett et al	1942	procaine	recording of action potential	mixed *Rana pipiens*	7.38	0.1–0.15 mM	0.02-0.03*)
Ehrenberg	1948	procaine	nerve muscle preparation**)	motor *Rana arvensis*	7.39		0.262
		xylocaine					0.815
Skou	1954	procaine	nerve muscle preparation	motor *Rana esculenta*	6.0	750–500 mg%	0.021–0.016*)
					7.0	150–100 mg%	0.047–0.032*)
					7.35	80–70 mg%	0.058–0.050*)
					8.0	40–30 mg%	0.09 –0.12 *)
Rud	1958	procaine	recording of action potential	mixed *Rana esculenta*	6.12		0.013*)
					7.08		0.021*)
					7.65		0.026*)
		Xylocaine			6.00		0.01*)
					7.10		0.11*)

*) base concentration calculated from equation (4) p. 27 $f_{BH^+} = 0.73$

**) minimum concentration calculated from diffusion theory

***) values from 12 hour experiments

DISCUSSION

Minimum concentration.

The minimum concentration of procaine was determined for the first time by GROS (1910) on the basis of clinical experiments carried out by HEINEKE and LÄWEN (1905). TREVAN and BOOCK (1927) used local anesthetics applied to the cornea of rabbits with the corneal reflex as indicator of anesthetic effect. BOEMINGHAUS and KOCHMANN (1929) determined the minimum concentration for procaine using a nerve-muscle preparation. To ensure that there was equilibrium between the anesthetic in and outside the nerve BOEMINGHAUS and KOCHMANN insist that the bathing time should be extended to 12–24 hours when low concentrations are used. It appears, however, from BOEMINGHAUS and KOCHMANN's own control experiments with a nerve in Ringer's for 24 hours, that such a long experimental time results in an increase in the threshold of the nerve to stimulation. With a higher threshold to stimulation it might be expected that the minimum concentration of the anesthetic would decrease. Therefore the experimental time should not be longer than necessary. In the experiments reported here the minimum concentration was determined which caused the action potential to disappear after 6–8 hours of exposure to the anesthetic. That this exposure time was considered sufficient to ensure equilibrium between the inner and outer solutions is based on the following considerations.

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2) In the experiments concerned with the time course of anesthesia the lowest xylocaine base concentration able to block a nerve was 0.15 mM. Extrapolating the time required to reduce the action potential to one per cent of its original value to lower base concentration a minimum concentration was found of the same order as that determined directly after 6–8 hours (*0.1 mM xylocaine base*). *At the minimum concentration* $t_{0.01}$ is infinite, i.e. $1/t_{0.01} = 0$. The minimum concentration was therefore determined by extrapolating to the concentration at which $1/t_{0.01} = 0$.

The experiments concerned with the determination of the minimum concentration were carried out on nerves which were 25 per cent thinner than the nerves used in the investigation of the whole course of anesthesia. Therefore, the blocking time in these experiments was about 60 per cent of that in the experiments on the time course (maximally about $2\frac{1}{2}$ hours). Thus, using an experimental exposure time of 6–8 hours there should be a reasonable safety margin that equilibrium be reached between the inner and

dency for solutions with high acid concentration to have a more rapid anesthetic effect. Comparison of experiments with different concentrations of the base component revealed that the anesthesia progressed more rapidly as the concentration of base increased. From this fact it is concluded that the course of anesthesia is for the most part determined by the concentration of base. This is in agreement with GROS (1910), TREVAN and BOOCK (1927), GARDNER, SEMB and GRAHAM (1931) and EHRENBERG (1948).

The fact that only the free base and not the acid is soluble in lipids (LOFGREN 1948, SKOU 1954) may account for the anesthetic action of the base component, since it can be bound to the lipids of the nerve fiber membrane.

The dependency of the minimum concentration on pH.

TREVAN and BOOCK (1927) and SKOU (1954) found that the minimum concentration of procaine base was lower at low than at high pH. The same was observed in the present experiments both for procaine and xylocaine. In the pH range 6–7 the same slope of pH effect for procaine was found as seen by SKOU (1954), but in the range of pH 7–8 a somewhat greater effect was seen (see Table 12).

That the minimum concentration varies with pH might be explained in various ways.

1) TREVAN and BOOCK (1927) assume that the buffer capacity of the nerve tissue causes the pH of the anesthetic solution within the nerve to approach that of the nerve. Thus with acid solutions the base concentration in the nerves should increase. The anesthetic effect would be more marked than expected from the outer base concentration. The reverse should obtain for anesthetic solutions at a pH higher than that of nerve tissue. SKOU's experiments indicate however that the buffer capacity of the nerve is not the only explanation after all. His experiments were carried out on a teased-out nerve and the pH was maintained constant by changing the anesthetic solution frequently. With this experimental technique it is unlikely that the nerve has a sufficiently large buffer capacity to change the pH. In the experiments reported here with intact nerves, an attempt was made to ensure that the nerve had the same pH as the anesthetic by immersing it in Ringer's solution at the pH to be used for an hour before the experiment. Since the minimum concentrations found show approximately the same pH dependency as found by SKOU (1954) it may be concluded that the effect of the buffer capacity of the nerve tissue is of little importance.

2) Since the protolysis of the anesthetic is pH dependent, a higher concentration of the acid component is required at low than at high pH to achieve

outer fluids even at concentrations only a few per cent above the minimum concentration.

Other authors have sought to avoid a long experimental time in determining the minimum concentration. SKOU (1954) removes the nerve sheath and separates the nerve into many fine threads so that an equilibrium between the inner and outer fluids is attained more rapidly. LUNDBERG (1948) used high concentrations and calculated the minimum concentration for procaine and xylocaine on the basis of the theory of free diffusion into the nerve. BENNETT et al. (1942) who used the decrease in amplitude of the action potential as measure of anesthetic effect, determined the minimum concentration as that concentration at which the amplitude was reduced at least 10 per cent and not more than 50 per cent within one hour. They found the same minimum concentration for procaine as that found in these studies. SKOU (1954) used the disappearance of a muscle contraction as measure of anesthetic effect in a nerve-muscle preparation and found a minimum concentration for procaine which was double the minimum concentration found in these studies at pH 6 and three times the present value at pH 7.3–8. SKOU (personal communication) suggests that the difference between the two studies may be that he uses a phosphate buffer whereas in this study a combination of phosphate and bicarbonate buffer was used.

It might furthermore be considered that SKOU records solely the response of the motor fibers, whereas the action potential in the study presented here is recorded from all fibers in the A group. The motor fibers belong to the α type of nerve fibers in the A group and are characterized by large diameter. The action potential contains also contributions from potentials from other A fibers (β, γ and δ) of smaller diameter than the α fibers. It is reasonable to suppose that there may be a spectrum of minimum concentrations corresponding to the various types of fiber. With the disappearance of the action potential as measure of anesthetic effect the minimum concentration is that of those fibers with the highest minimum concentration. These are probably α fibers corresponding to GASSER's (1943) assumption that the blocking time is proportional with fiber diameter. In this case the minimum concentration was determined for the same fibers in SKOU's as in the present studies.

The experiments contribute no information as to the breadth of such a spectrum of minimum concentrations if such a spectrum exists. To determine the minimum concentration in a whole nerve for the most sensitive fibers, one should in principle find the smallest concentration which just serves to diminish the action potential amplitude. This procedure is however not practicable since as mentioned on page 93 concentrations under the minimum concentration reduce the action potential amplitude of the single nerve fibers.

Base concentration versus acid concentration as anesthetic agent.

Comparing the time course of anesthesia in experiments at the same base concentration but with different concentrations of acid, there was no ten-

Anesthesia advanced rapidly at first and then more slowly. For the lowest xylocaine base concentrations investigated (0.05–0.16 mM) it took on the average about 10 minutes for the amplitude to fall to half and for 0.15–0.16 mM solutions about 70 minutes to achieve full block. With increasing concentrations of xylocaine base the nerve was anesthetized more rapidly. This was especially marked in the range of concentrations up to about 1 mM xylocaine base. In the concentration range 1.0–1.5 mM the time to half amplitude was about 1 minute and the blocking time about 8 minutes. For the higher xylocaine base concentrations investigated (8.7–13.7 mM) the time to half amplitude was on the average about one half minute and the time to full block about 4 minutes. Thus, whereas the course of anesthesia progressed about 10 times more rapidly at a concentration 10 times above the lowest concentration, it was only about twice as rapid with a 10 times increase of large concentrations.

In the case of procaine there were not sufficient experiments to allow a detailed description of the couse of anesthesia as a function of concentration. The tendency is, however, the same as that described above for xylocaine (Fig. 21).

Comparison of the time course of anesthesia with xylocaine and procaine

Since most experiments with procaine were performed with 1 mM procaine base, the course of anesthesia will be compared with xylocaine at the same concentration. The time to half amplitude was about 60 per cent and the time to block about 40 per cent longer for xylocaine than for procaine (Figure 20 and Table 6). It may be questioned whether the more rapid anesthetic effect of procaine than of xylocaine at the same base concentration may be related to the fact that procaine has a smaller minimum concentration. When the base concentration is expressed in units of the minimum concentration (C_B/C_m = 40) the time to half amplitude was twice as long for procaine as for xylocaine and the blocking time was one and a half times as long (Figure 22 A, B and Table 6). In other words, xylocaine acts more rapidly than procaine when the substances are compared at base concentrations which are the same multiple of the minimum concentration. The time course of anesthesia can therefore not be explained solely in terms of the concentration of the active anesthetic component (base concentration) or the minimum concentration, but must depend on other factors as well, as discussed in chapter 5.

Ehrenberg (1948) investigated the time to block for xylocaine and procaine using a nerve muscle preparation. The xylocaine experiments were

the same base concentration It might therefore be argued that the acceleration of the anesthetic effect with decreasing pH for the same base concentration in the *region of small concentrations must be due to the fact th* acid component also has anesthetic *properties This problem was discussed* on page 69 where it was demonstrated that if the acid component has an *anesthetic effect it must be* considerably less than that of the base component

3) A third possible explanation for the fact that the minimum concentration of the base decreases with decreasing pH would be a pH dependence of the susceptibility of the nerve to anesthesia A decrease in pH is associated with an increased threshold to stimulation (ADRIAN 1920, LEHMANN 1937 a) Such an increase in threshold in an anesthetized nerve, in which the membrane current is reduced and is barely sufficient to stimulate the adjacent section of the nerve, would be expected to cause a block at a lower concentration of the anesthetic

That the susceptibility of the nerve to local anesthetic is pH dependent might also be explained on the basis of the mechanism by which local anesthetics are assumed to block the nerve impulse It appears likely that the local anesthetic blocks the nerve fiber by reducing the permeability of its membrane to sodium (see p 13) Permeability to sodium is likewise reduced when calcium ions are bound to the membrane, that is, calcium acts synergistically with local anesthetics (see page 11) The assumption has been made, that calcium is bound more firmly to the nerve membrane *in an acid than in an alkaline medium* (DETTBARN and STAMPFLI 1957) A given concentration of calcium and local anesthetic should therefore be *most effective at low pH and the minimum concentration* would be expected *to be lower at low* pH

4) An explanation for the fact that the minimum concentration increases with increasing pH might be that a certain concentration of anesthetic must *be bound* to the nerve membrane *in order to block it* SKOU (1951) found that a local anesthetic is bound to nerve tissue partially by adsorption and partially by solution of the base component in the dry phase of the nerve chiefly in the lipids The partition coefficient of *the base component of* procaine between the dry and fluid phases of nerve diminished with increasing pH (SKOU 1951) Therefore, to obtain that a given amount of base be bound to the dry phase of nerve demands more *base* at *high than at low pH*

The time course of anesthesia.

With the amplitude of the action potential as measure *it was possible to* follow the time course of anesthesia from the application of the anesthetic to full nerve block

discussed in the literature. LORENTE DE NÓ (1950) doubts the value of such experiments, because in his opinion removal of the sheath damages the fibers and alters the characteristics. He concludes that it has not been shown that the nerve sheath itself delays anesthesia. Against LORENTE DE NÓ's arguments it may be said that he himself (1950) found that nerve roots lacking an epineurium and having only an endoneurium were blocked about 10 times more rapidly by a sodium free solution than a nerve with sheath. NORDQUIST (1952 b) instead of dissecting the sheath away subjected the nerve to the action of hyaluronidase which dissolves the connective tissue so diffusion through it occurs more easily. Applying procaine to a nerve treated in this way he found that the blocking time was diminished to about half. In the case of nerves without sheath hyaluronidase had no effect on the action of procaine. CRESCITELLI (1951) reported a more rapid action of antihistamine on nerves without sheath. By washing the substances out of the nerve again by "rolling" the sheath back in place and repeating the experiment with sheath he found that the blocking time again approached the values in the intact nerve. He concluded therefore that removal of the sheath does not alter the characteristics of the fibers (see also p. 91).

carried out with base concentrations of from 0.85 to 1.8 mM (pH 7.39). Corresponding blocking times varied from 3000 to 700 seconds (with the exception of one extreme value of 5640 seconds with $C_B = 0.89$ mM). In the experiments described here (Figure 20) the blocking times in the same concentration range varied from about 900 to 100 seconds. In the case of procaine, the smallest base concentration (0.27 mM) used by EHRENBERG (1948) gave a blocking time of 3840 seconds and the highest concentration (0.63 mM) gave a blocking time of 660 seconds. Compared with the experiments in my study (Figure 21) blocking times of 700 and 100 seconds would be expected.

There is no clear explanation for the difference in blocking times in EHRENBERG's and in my experiments. The only difference in technique which might be of importance is that EHRENBERG did not use carbon dioxide bicarbonate buffer whereby the minimum concentration might be increased (SKOU, personal communication). It does not however seem likely that this can be the whole explanation for the difference in blocking times.

The importance of the sheath for the time course of anesthesia.

The importance of the sheath for the time course of anesthesia has often been investigated by comparing the anesthesia of nerves with and without sheath. I have found that a nerve stripped of its sheath is blocked about 2–4 times as rapidly by xylocaine as a nerve with intact sheath. This is in agreement with TASAKI (1953) who found that removal of the sheath reduced the blocking time to half or less (urethane). In a short abstract FRUMENT and LANZONI (1952) described that xylocaine, procaine and other local anesthetics blocked the action potential 8–10 times more rapidly when the sheath was removed. In other investigations of local anesthetic action on nerves with and without sheath the experimental conditions were quite different. *SKOU (1954) investigated the effect of various local anesthetics on nerves in a nerve-muscle preparation using muscle contraction to indicate passage of the nerve impulse.* By removing the sheath and separating the nerve into many fine threads, the time for blocking of the motor fibers was diminished about 10 times as compared with intact nerves. This difference is not surprising since it is known that local anesthetics applied to single nerve fibers block conduction almost instantaneously (KATO 1936, TASAKI and TAKEUCHI 1942 and TASAKI 1953). FENG and LIU (1949) found a still greater difference at 25 and 4°C in that cocaine blocked the nerve action potential 35–50 times faster without than with sheath.

The value of experiments carried out on sheathless nerves has been much

discussed in the literature. LORENTE DE NÓ (1950) doubts the value of such experiments because in his opinion removal of the sheath damages the fibers and alters the characteristics. He concludes that it has not been shown that the nerve sheath itself delays anesthesia. Against LORENTE DE NÓ's arguments it may be said that he himself (1950) found that nerve roots lacking an epineurium and having only an endoneurium were blocked about 10 times more rapidly by a sodium free solution than a nerve with sheath. NORDQUIST (1952 b) instead of dissecting the sheath away subjected the nerve to the action of hyaluronidase which dissolves the connective tissue so diffusion through it occurs more easily. Applying procaine to a nerve treated in this way, he found that the blocking time was diminished to about half. In the case of nerves without sheath hyaluronidase had no effect on the action of procaine. CRESCITELLI (1951) reported a more rapid action of antihistamine on nerves without sheath. By washing the substances out of the nerve again by "rolling" the sheath back in place and repeating the experiment with sheath he found that the blocking time again approached the values in the intact nerve. He concluded therefore that removal of the sheath does not alter the characteristics of the fibers (see also p. 91).

Chapter 5

COMPARISON OF THE EXPERIMENTAL TIME COURSE OF ANESTHESIA WITH THAT PREDICTED FROM FREE DIFFUSION OF THE ANESTHETIC INTO THE NERVE

The time course of anesthesia in a whole nerve was found to differ from that of a single nerve fiber in which block occurs instantaneously. Hence the time course of anesthesia in a whole nerve is determined at any rate in part by diffusion into the nerve. This was assumed in earlier investigations by Bennett et al. (1942) and by Ehrenberg (1948) who, by use of diffusion theory, calculated the minimum concentration and the diffusion coefficient for the anesthetic into a nerve

In this chapter a more detailed discussion will be given of the extent to which it is possible to describe the time course of xylocaine anesthesia in a peripheral nerve on the basis of the theory of free diffusion Obviously in a whole nerve there is *nothing resembling free diffusion in a homogeneous* medium In the first place the nerve is surrounded by a sheath, in the second place the anesthetic can only penetrate the interfibrillar spaces of the nerve; *finally the diffusion process is complicated by the fact that the anesthetic is* bound to the nerve tissue as it penetrates into the nerve and this implies that one is not dealing with a diffusion process which can be described by *using the simple Fick law of diffusion However, under certain conditions* diffusion can progress qualitatively in the same way as free diffusion even if the diffusing substance undergoes a physical or chemical reaction in the course of its diffusion (see page 99) *In this case diffusion would progress* more slowly, as would be evidenced by a smaller diffusion coefficient than in free diffusion The inhomogeneity of the nerve would likewise delay diffusion, the area through which the substance diffuses *into the nerve being* less than the total surface area of the nerve To differentiate the diffusion coefficient in a homogenous medium from that in an inhomogenous medium, the latter can be described as the *effective diffusion coefficient* (Shanes and Berman 1955) In the discussion below, the diffusion coefficient in nerve is used in the sence of *apparent* or *effective* diffusion coefficient

EHRENBERG's (1948) calculation of the minimum concentration and diffusion coefficient from diffusion theory are based solely on the blocking time. The premise for this procedure is that the time course of anesthesia can be described from the theory of free diffusion, i.e. that the factors which complicate diffusion in a nerve do not invalidate the applicability of the theory of free diffusion to account for the course of anesthesia. EHRENBERG has not stated to what extent this premise has been fulfilled and it is uncertain to what extent it is justified to determine minimum concentration and diffusion coefficient from the blocking time alone. The material presented in my study, where the time course of anesthesia was determined at various concentrations and where a separate experimental determination of the minimum concentration was carried out, should be suitable to investigate to what extent a *simple diffusion concept* can account for the diminution in action potential amplitude and whether the minimum concentration calculated on this basis is identical with that determined experimentally.

The mathematical basis for calculation of diffusion of the anesthetic into nerve assuming free diffusion

Let the nerve at time zero be placed in a large volume of a solution of the local anesthetic at concentration C_e. Considering the nerve as an infinitely long homogeneous cylinder, the diffusion of the anesthetic *into* and within the nerve will be determined by the following equation

$$\frac{\partial^2 C}{\partial r^2} + \frac{1}{r}\frac{\partial C}{\partial r} = \frac{1}{D}\frac{\partial C}{\partial t} \quad 0 \leq r \leq r_0,\ t \geqq 0 \tag{1}$$

with the boundary conditions

$$C = 0 \text{ for } t = 0 \text{ and } 0 \leq r < r_0$$

and

$$C = C_e \text{ for } r = r_0 \text{ and } t \geqq 0$$

$C = C(r, t)$ indicates the concentration of anesthetic at a distance r from the nerve axis at time t, whereas D is the diffusion coefficient of the anesthetic in the nerve and r_0 is the radius of the nerve.

Solving equation (1) the following expression for the variation of the concentration of anesthetic through the nerve is obtained (compare for instance CARSLAW and JAEGER 1947)

$$C(r, t) = C_e\left\{1 - 2\sum_{n=1}^{\infty} e^{-\frac{D\beta_n^2 t}{r_0^2}} \frac{J_0\left(\frac{r\beta_n}{r_0}\right)}{\beta_n J_1(\beta_n)}\right\}, \tag{2}$$

where J_0 and J_1 are Bessel functions and β_n's are the roots $J_0(\beta_n) = 0$

CHAPTER 5

COMPARISON OF THE EXPERIMENTAL TIME COURSE OF ANESTHESIA WITH THAT PREDICTED FROM FREE DIFFUSION OF THE ANESTHETIC INTO THE NERVE

The time course of anesthesia in a whole nerve was found to differ from that of a single nerve fiber in which block occurs instantaneously. Hence the time course of anesthesia in a whole nerve is determined at any rate in part by diffusion into the nerve. This was assumed in earlier investigations by BENNETT et al. (1942) and by EHRENBERG (1948) who, by use of diffusion theory, calculated the minimum concentration and the diffusion coefficient for the anesthetic into a nerve.

In this chapter a more detailed discussion will be given of the extent to which it is possible to describe the time course of xylocaine anesthesia in a peripheral nerve on the basis of the theory of free diffusion. Obviously in a whole nerve there is nothing resembling free diffusion in a homogeneous medium. In the first place the nerve is surrounded by a sheath, in the second place the anesthetic can only penetrate the interfibrillar spaces of the nerve; finally the diffusion process is complicated by the fact that the anesthetic is bound to the nerve tissue as it penetrates into the nerve and this implies that one is not dealing with a diffusion process which can be described by using the simple Fick law of diffusion. However, under certain conditions diffusion can progress qualitatively in the same way as free diffusion even if the diffusing substance undergoes a physical or chemical reaction in the course of its diffusion (see page 99). In this case diffusion would progress more slowly as would be evidenced by a smaller diffusion coefficient than in free diffusion. The inhomogeneity of the nerve would likewise delay diffusion, the area through which the substance diffuses into the nerve being less than the total surface area of the nerve. To differentiate the diffusion coefficient in a homogenous medium from that in an inhomogenous medium the latter can be described as the "effective diffusion coefficient" (SHANES and BERMAN 1955). In the discussion below, the diffusion coefficient in nerve is used in the sence of *apparent* or *effective* diffusion coefficient.

EHRENBERG's (1918) calculation of the minimum concentration and diffusion coefficient from diffusion theory are based solely on the blocking time. The premise for this procedure is that the time course of anesthesia can be described from the theory of free diffusion, i. e. that the factors which complicate diffusion in a nerve do not invalidate the applicability of the theory of free diffusion to account for the course of anesthesia. EHRENBERG has not stated to what extent this premise has been fulfilled and it is uncertain to what extent it is justified to determine minimum concentration and diffusion coefficient from the blocking time alone. The material presented in my study, where the time course of anesthesia was determined at various concentrations and where a separate experimental determination of the minimum concentration was carried out, should be suitable to investigate to what extent a *simple diffusion concept* can account for the diminution in action potential amplitude and whether the minimum concentration calculated on this basis is identical with that determined experimentally.

The mathematical basis for calculation of diffusion of the anesthetic into nerve assuming free diffusion.

Let the nerve at time zero be placed in a large volume of a solution of the local anesthetic at concentration C_e. Considering the nerve as an infinitely long homogeneous cylinder, the diffusion of the anesthetic into and within the nerve will be determined by the following equation

$$\frac{\partial^2 C}{\partial r^2} + \frac{1}{r}\,\frac{\partial C}{\partial r} = \frac{1}{D}\,\frac{\partial C}{\partial t}, \quad 0 \leqq r \leqq r_0 \quad t \geqq 0 \tag{1}$$

with the boundary conditions

$$C = 0 \text{ for } t = 0 \text{ and } 0 \leqq r < r_0$$

and

$$C = C_e \text{ for } r = r_0 \text{ and } t \geqq 0$$

$C = C(r, t)$ indicates the concentration of anesthetic at a distance r from the nerve axis at time t whereas D is the *diffusion coefficient* of the anesthetic in the nerve and r_0 is the radius of the nerve.

Solving equation (1) the following expression for the variation of the concentration of anesthetic through the nerve is obtained (compare for instance CARSLAW and JAEGER 1947)

$$C(r, t) = C_e \left\{ 1 - 2 \sum_{n=1}^{\infty} e^{-\frac{D\beta_n^2 t}{r_0^2}} \frac{J_0\left(\frac{r\beta_n}{r_0}\right)}{\beta_n J_1(\beta_n)} \right\}, \tag{2}$$

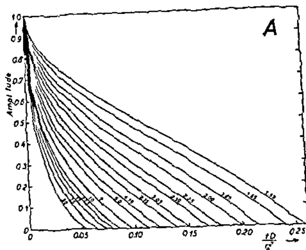
where J_0 and J_1 are Bessel functions and β_n's are the roots $J_0(\beta_n) = 0$

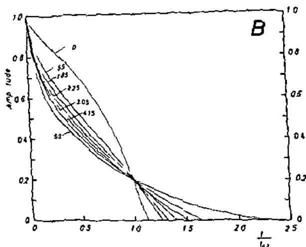
Fig. 25 The concentration distribution in a cylinder at various times after its placement in a medium containing a diffusible substance of constant concentration (C_e). The initial concentration in the cylinder is zero. The different curves give the concentration within the cylinder as a function of the distance from the axis at different times indicated on the curves in terms of Dt/r_0^2, where D is the diffusion coefficient (CARSLAW and JAEGER 1947).
Ordinate: concentration in units of the outer concentration (C_e).
Abscissa: distance from the axis in units of the radius (r_0).

In Figure 25 these distributions of concentrations at various times are demonstrated graphically.

It follows from expression (2) that the time required to obtain a certain fraction of the outer concentration C_e at distance r from the axis of the nerve is inversely proportional with the diffusion coefficient D of the anesthetic and proportional with the square of the nerve radius r_0. Hence, for various anesthetics the course of anesthesia will be the faster the greater the diffusion coefficient in the nerve.

From the distribution of concentrations as given by (2) and Figure 25 and with the assumptions that there exists a minimum concentration which is the same for all nerve fibers in the A group and that the action potential amplitude is proportional with that area in the nerve where the concentration is less than the minimum concentration, the time course of the diminution in amplitude can be predicted. In Figure 26 A this is shown for different values of the ratio between the outer concentration C_e and the minimum concentration C_m, the time being expressed in arbitrary units as Dt/r_0^2. It is seen that the diminution in action potential amplitude occurs more





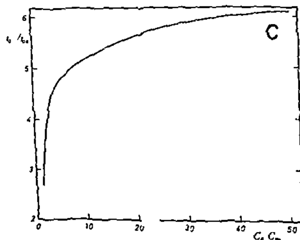


Fig 26 A The theoretical time course of anesthesia in a nerve with various outer concentrations (C_e) The figures on the curves give the concentration in units of the minimum concentration (C_m) (Constructed from Fig. 25)

Ordinate calculated action potential amplitude in units of the initial value

Abscisse time in terms of Dt/r_0^2 where D is the diffusion coefficient, t time in seconds and r_0 nerve radius in centimeters

B To compare the relative time course of anesthesia with various outer concentrations the calculated curves from (A) have been normalized to intersect at amplitude 0 2

Ordinate calculated action potential amplitude in units of the initial value

Abscisse time after application of the local anesthetics in units of the time required to reduce the amplitude to 0 2.

C The ratio $t_{0.2}/t_{0.6}$ (ordinate) for the calculated time courses of anesthesia as a function of the outer concentration (C_e) in units of the minimum concentration (abscissa) $t_{0.2}$ and $t_{0.6}$ are the times required to reduce the action potential amplitude to 20 and 60 per cent of its initial value, respectively

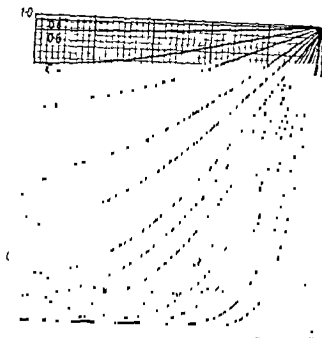


Fig 25 The concentration distribution in a cylinder at various times after its placement in a medium containing a diffusible substance of constant concentration (C_e) The initial concentration in the cylinder is zero The different curves give the concentration within the cylinder as a function of the distance from the axis at different times indicated on the curves in terms of Dt/r_0^2, where D is the diffusion coefficient (CARSLAW and JAEGER 1947) Ordinate concentration in units of the outer concentration (C_e) Abscissa distance from the axis in units of the radius (r_0)

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experiments was known C_m the minimum concentration could be calculated. With such a determination of the minimum concentration both the theory and the experimental error in the determination of $t_{0.2}/t_{0.6}$ limited the range of concentrations at which the determination could be carried out with reasonable certainty. As regards the theory it is apparent from Figure 26 B that the relative time course varied less with the outer concentration as this became progressively larger.

An increase in outer concentration from 1.5 to 3 times the minimum concentration was accompanied by a greater change in the relative time course than an increase in concentration from 3 to 50 times the minimum concentration. Furthermore, in the range of concentration 4–50 times the minimum concentration, the relative time course varied only a few per cent in the amplitude range 100–20 per cent. Thus, on theoretical grounds alone the relative time course was less suitable to determine the minimum concentration as the outer concentration increased (Fig. 26 C). The experimental error in the determination of the ratio $t_{0.2}/t_{0.6}$ increased similarly with increasing outer concentrations of anesthetic. With a concentration about two times the minimum concentration the error was about ± 1 per cent, with 10 times the minimum concentration it was ± 2 per cent and with 50 times the minimum concentration it was ± 5 per cent. With these experimental errors the uncertainty in the determination of the minimum concentration was, according to the theoretical correlation between C_{II}/C_m and $t_{0.2}/t_{0.6}$ (Fig. 26 C), ± 2 per cent with an outer concentration two times the minimum concentration, ± 15 per cent at 10 times the minimum concentration and ± 60 per cent with 50 times the minimum concentration. A determination of the minimum concentration from the experimental time course of anesthesia was therefore only possible with reasonable certainty by applying concentrations less than about 10 times the minimum concentration.

The calculated and the experimentally determined minimum concentrations for the 16 experiments with xylocaine on whole nerve are presented in Table 13. With low outer concentrations the calculated minimum concentration was equal to or less than that found experimentally and with increasing outer concentrations the calculated values were greater than those found experimentally.

In the range of outer concentrations up to four times the minimum concentration the experimentally and theoretically determined minimum concentrations agreed to within 30 per cent in 7 of 10 experiments and in the remaining three experiments (outer concentration less than $2\ C_m$) the calculated minimum concentration was 3–5 times less than the experimental. In 6 experiments with outer concentrations of more than four times the

rapidly as the outer concentration C_e of the anesthetic increases To illustrate the effect of the outer concentration on the shape of the curves the relative time courses are compared in Figure 26 B in that all curves have been normalized to pass through the same point of amplitude 0 2 The relative time course depends on the ratio C_e/C_m in that the last phase of anesthesia occurs relatively most rapidly when the outer concentration of the anesthetic is small

The criteria to determine whether the experimental time course of anesthesia with different concentrations and in nerves of various thickness can be described in mathematical terms of free diffusion are therefore that

1) there is agreement between experimental and theoretical time courses in individual experiments,
2) the calculated minimum concentrations are identical with those found experimentally,
3) the same diffusion coefficient is obtained in experiments with different concentrations of the local anesthetic

The calculated time course of anesthesia as compared with the experimental

To compare the theoretical and experimental time course of anesthesia the theoretical curve was selected from Figure 26 A whose form was similar to the experimental This was found as the theoretical curve which had the same ratio between times to 20 per cent ($t_{0\,2}$) and 60 per cent ($t_{0\,6}$) of the initial action potential amplitude as the experimental curve (Fig 26 C) To test the agreement between this curve and the course of the experimental curve, the time axis of the theoretical curve was adjusted by determining D in the expression Dt/r_0^2 in such a way that the best possible agreement is obtained between the two time courses

In this way it was found possible in the case of experiments with *xylocaine* over the whole range of concentrations used to obtain such a close agreement between theoretical and experimental anesthetic course that the maximal deviation did not exceed 5 per cent of the initial amplitude in the range of amplitude from 100 to 15 per cent Below an amplitude of 15 per cent the experimental diminution in amplitude occurred in general slower than calculated from free diffusion

The minimum concentration calculated from the theory of free diffusion.

The theoretical course of anesthesia in Figure 26 A selected according to the ratio $t_{0\,2}/t_{0\,6}$ similar to the experimental course was characterized by the ratio between C_B ($= C_e$) and C_m Since the base concentration C_B used in the

experiments was known, C_m, the minimum concentration could be calculated With such a determination of the minimum concentration both the theory and the experimental error in the determination of $t_{0\,2}/t_{0\,6}$ limited the range of concentrations at which the determination could be carried out with reasonable certainty As regards the theory, it is apparent from Figure 26 B that the relative time course varied less with the outer concentration as this became progressively larger

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The calculated and the experimentally determined minimum concentrations for the 46 experiments with xylocaine on whole nerve are presented in Table 13 With low outer concentrations the calculated minimum concentration was equal to or less than that found experimentally and with increasing outer concentrations the calculated values were greater than those found experimentally

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In this way it was found possible in the case of experiments with *xylocaine over the whole range of concentrations used to obtain such a close agreement* between theoretical and experimental anesthetic course that the maximal deviation did not exceed 5 per cent of the initial amplitude in the range of amplitude from 100 to 15 per cent Below an *amplitude of 15 per cent the* experimental diminution in amplitude occurred in general slower than calculated from free diffusion

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minimum concentration the minimum concentrations calculated from diffusion theory were on the average eight times greater than the directly determined values (extreme value 1.1 and 31 times).

The fact that the error in the calculated values is relatively greater when the concentration is high can explain a wide variability with high outer concentrations. It can however not explain the systematic difference between the calculated and the directly determined minimum concentrations with small and large concentrations nor can it explain the wide variability with outer concentrations as low as four times the minimum concentration. The feasibility of determining the minimum concentration from the time course of anesthesia with outer concentrations up to four times the minimum concentration is uncertain since there were only 10 experiments in this range.

The diffusion coefficient calculated simultaneously with the minimum concentration from the theory of free diffusion

The time axis for the theoretical decay in action potential amplitude which had a shape similar to that of the experimental curve was adjusted by determining the value for the diffusion coefficient which gave the best agreement between experimental and theoretical time course (cf. p. 88). The diffusion coefficients determined in this way for the 46 experiments with xylocaine on whole nerve are given in Table 13, column 7. With base concentrations of 1.5–4 times the minimum concentration the calculated diffusion coefficient varied in the 10 experiments from 1.0–3.7 × 10^{-7} cm²/sec. With base concentrations of 4–90 times the minimum concentration the calculated diffusion coefficient averaged 8.7 × 10^{-7} cm²/sec with a variation from 2 to 23 × 10^{-7} cm²/sec. Two extreme values (0.7 and 40 × 10^{-7} cm²/sec) were not included in the calculation of mean value. That the diffusion coefficients corresponding to base concentrations greater than four times the minimum concentration showed such wide variability was a result of the widely varying calculated minimum concentrations and no real significance can therefore be attached to them.

Conclusion. As concerns the agreement between the experimental and the theoretical time course in experiments with *xylocaine* agreement was obtained within the range of action potential amplitudes from 100 to 15 per cent of the initial value by suitable selection of minimum concentration and diffusion coefficient. With lower amplitudes the experimental time course was slower than the theoretical.

As concerns the comparison between the experimentally found and the calculated minimum concentrations there was no agreement in most experi-

Table 13

Experimental and calculated minimum concentration and diffusion coefficient for xylocaine

experiment no	base concentration C_B mM	pH	experimental minimum concentration $C_{m(exp)}$ *) mM	$\frac{C_B}{C_{m(exp)}}$	calculated minimum concentration $C_{m(calc)}$ mM	diffusion coefficient cm²/sec × 10 7	$\frac{C_{m\,calc}}{C_{m\,exp}}$
156	0 15	6 88	0 10	1 5	0 10	1 4	1 0
115	0 15	6 88	0 10	1 5	0 02	1 0	0 2
123	0 16	6 90	0 10	1 6	0 08	1 7	0 8
124	0 20	7 00	0 105	1 9	0 028	1 0	0 3
126	0 20	7 02	0 105	1 9	0 02	1 0	0 2
116	0 24	7 09	0 11	2 2	0 10	1 5	0 9
90	0 30	6 77	0 09	3 3	0 117	3 2	1 3
92	0 33	6 84	0 094	3 5	0 10	3 7	1 1
136	0 33	6 84	0 094	3 5	0 10	3 3	1 0
91	0 41	6 92	0 10	4 1	0 09	3 4	0 9
93	0 48	6 98	0 103	4 7	0 26	7 5	2 5
106	0 48	6 38	0 065	7 4	0 28	12 2	4 3
107	0 63	6 50	0 072	8 75	0 29	7 8	4 0
86	0 63	7 11	0 11	5 7	0 30	7 8	2 7
94	0 85	7 28	0 122	7 0	0 36	5 2	3 0
84	0 87	7 29	0 122	7 1	0 31	4 3	2 5
99	0 96	6 70	0 085	11 3	0 21	3 0	2 4
157	1 0	6 70	0 085	11 75	0 40	7 6	4 7
158	1 0	6 70	0 085	11 8	0 45	12 9	5 3
87	1 4	7 54	0 14	10 0	0 16	2 7	1 1
88	1 4	7 55	0 14	10 0	0 21	3 3	1 5
100	1 5	6 89	0 097	15 4	0 78	12 6	8 0
101	1 59	6 92	0 10	15 9	0 32	2 3	3 2
76	1 62	6 93	0 10	16 2	0 57	10 0	5 7
95	1 74	6 95	0 10	17 4	1 08	18 0	10 8
96	1 78	6 97	0 10	17 8	0 60	6 1	6 0
103	1 8	7 01	0 105	17 2	0 69	9 0	6 1
128	1 8	6 99	0 105	17 2	0 27	6 3	2 6
154	2 0	7 02	0 105	19 1	0 50	6 7	4 8
109	2 63	6 83	0 093	28 2	1 15	14 8	12 4
159	3 0	6 90	0 10	30 0	0 40	4 9	4 0
104	3 1	7 23	0 12	25 8	1 21	11 4	10 3
79	3 47	7 30	0 123	28 2	0 07	2 3	0 6
108	4 36	7 44	0 132	33 6	0 17	3 9	1 3
130	4 47	7 09	0 11	10 6	0 60	7 3	5 5
110	4 78	7 12	0 112	42 7	0 24	4 0	2 1
111	5 13	7 16	0 115	46 7	2 0	12 4	17 0
161	5 5	7 57	0 14	39 3	3 40	10 0	24 0
98	6 3	7 66	0 147	42 8	0 38	3 5	2 6
97	6 31	7 64	0 116	43 2	0 73	5 8	5 4
160	8 0	7 40	0 13	61 6	3 60	20 6	27 7
112	8 71	7 44	0 132	67 0	0 60	7 1	4 5
114	9 55	7 49	0 136	70 8	2 58	16 2	11 0
113	10 0	7 52	0 138	72 4	2 80	23 5	20 0
162	13 7	7 72	0 152	90 0	1 70	12 5	31 0
(102	0 19	5 67	0 018	10 5	0 002	0 7	0 1)

*) Values from Fig 18

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As concerns the comparison between the experimentally found and the calculated minimum concentrations there was no agreement in most experi-

ments In addition to the fact that the calculated minimum concentrations varied widely there was a systematic disagreement in that the calculated minimum concentrations were higher as the outer concentrations increased

Finally, as concerns the diffusion coefficient this varied in the various experiments by a factor of ten

Hence the model of free diffusion of the anesthetic into the nerve cannot be used to calculate minimum concentrations which agree with the experimentally determined minimum concentrations and the diffusion coefficients in different experiments calculated therefrom are not identical

FACTORS COMPLICATING THE DESCRIPTION OF THE TIME COURSE OF ANESTHESIA IN TERMS OF FREE DIFFUSION

The analysis of the mathematical expression describing free diffusion into *the nerve indicated, that it should be possible from the time course of anesthesia* with concentrations up to 10 times the minimum concentration to calculate an effective diffusion coefficient and minimum concentration *if there were no other conditions in the nerve invalidating the use of this expression* This seems, however, to be the case in that it was not possible in this range of concentrations to calculate minimum concentrations identical with those *found experimentally An attempt is made below to evaluate the complicating* factors which could invalidate the use of the theory for free diffusion into nerve

Action potential amplitude as a measure of the number of active nerve fibers.

Even if there is no direct proof it is usually assumed that the action potential amplitude is proportional to the number of active nerve fibers This assumption was made use of, for example, in GASSER and ERLANGER's (1937) reconstruction of the action potential of a whole nerve by summation of the potentials of individual nerve fibers

The effect of subminimal concentrations of anesthetic on action potential amplitude.

If it is assumed that the action potential amplitude is proportional with that area of the nerve in which the concentration of the local anesthetic is less than the minimum concentration, it must also be assumed that the minimum concentration has an all or none effect on action potential amplitude, such that concentrations above the minimum concentration block the action potential instantaneously, whereas subminimal *concentrations have* no effect on the amplitude In fact TASAKI and TAKEUCHI (1942) found that

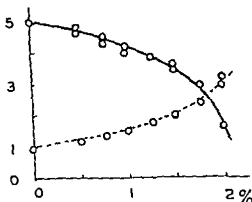


Fig 27 The effect of subminimal concentrations of urethane on a single node in a frog nerve fiber (TASAKI and TAKEUCHI 1942) The action current (upper curve) and the threshold of stimulation (lower curve) as a function of concentration
Ordinate threshold in units of the threshold in normal nerve The normal size of action current is taken as being equivalent to five times the normal threshold
Abscissa the concentration of urethane in per cent

urethane in subminimal concentrations diminished the action potential amplitude and conduction velocity and increased the threshold to stimulation of single nerve fibers (Figure 27) Subminimal concentrations of cocaine, which should be more comparable with xylocaine and procaine than urethane, exert the same effect although the effective concentration range is considerably less for cocaine than for urethane (TASAKI 1958)

The effect of subminimal concentrations of xylocaine and procaine on the time course of anesthesia could only be estimated roughly, but the decrease in amplitude occurred more rapidly in the beginning than was calculated whereas there was no effect on blocking time The relative time course at the end of anesthesia should therefore be slower than to be expected from the theoretical curves of Fig 26 A This effect would be greatest with the smallest concentrations

The nerve considered as an homogeneous circular cylinder.

Measurements of the diameter of nerve demonstrated that at the points subjected to the anesthetic it may be considered circular in cross section Furthermore no differences in nerve diameter were found within the 10 mm exposed to the anesthetic nearest to the nerve s peripheral end, i e where the nerve was thinnest

On the other hand the nerve is not homogeneous since there is a nerve sheath and nerve fibers surrounded by interstitial fluid

The nerve sheath as diffusion barrier.

The importance of the nerve sheath as diffusion barrier has been a subject for disagreement. SHANES (1951) attempted to demonstrate the *role* of the sheath directly by filling stripped off sheath with a solution containing radioactive sodium and closing the ends. For comparison a whole nerve was saturated with radioactive sodium and thereafter the diffusion was measured as it occurred into a Ringer's bath in both cases. The time for diffusion of sodium was practically the same through the sheath alone as through the intact nerve, and SHANES concludes that it is the sheath alone which constitutes the diffusion barrier.

According to LORENTE DE NO (1950) the sheath in itself presents no resistance to diffusion. Its effect is that it packs nerve fibers and endoneurium so closely together that penetration is rendered difficult. This assumption is supported by experiments which indicate that sheathless nerves placed for 2–4 hours in Ringer's solution increase in weight in contrast to intact nerves which do not increase in weight. According to FENG and LIU (1949) this increase is 10 per cent and according to SHANES (1953) it may amount to up to 40 per cent. Correspondingly, TRUANT and LANZONI (1952) and FRANKENHAEUSER and NYSTROM (1951) found that nerves without sheath increase in diameter when placed in Ringer's solution. *In my experiments* the increase in diameter averaged 6 per cent in the course of one hour. NYSTROM and SODERBERG (1955) *found that the increase in diameter depends* on the hydrogen ion concentration and is maximal at pH 5.

The findings described on page 73 indicate that the sheath has a delaying action on diffusion even if the results from experiments on nerves without sheath might be affected by changes in the nerve due to removal of the sheath.

In the xylocaine experiments on nerves *with* sheath it was found that the time course of anesthesia could not be described by the mathematics of free diffusion. *If the cause for this were the complicating effect of the sheath* on the course of diffusion, it should be possible to obtain agreement with the theory in nerves without sheath. This has not been possible. It has, it is true, been possible as in experiments with nerves with sheath, to obtain agreement between experimental and theoretical time course, but the minimum concentrations calculated thereby varied in the same way as in the case of experiments with nerves with sheath. The presence of the sheath can therefore not account for the insufficiency of simple *diffusion theory to* describe the time course of anesthesia.

The dependence of diffusion on the presence of nerve fibers and their distribution

When the distal part of the nerve is stimulated the anesthetized part of the nerve which determines the time course of anesthesia is the thinnest 10 mm (cf p 54) segment closest to the pre-anesthetic electrode (L_{1})

Within the distal part of the sciatic nerve including the distal 15 mm of the anesthetized portion of the nerve there is only one small branch containing 8 10 fibers Ramus profundus anterior and posterior of the sciatic nerve leave about sixteen mm proximal to the point at which the distal portion of the nerve contacts the local anesthetic These gross anatomical findings were confirmed by serial sections of the nerve with 0 5 mm between sections*) Therefore the number of fibers must be assumed to be practically identical at the point of stimulation and at the distal part of the nerve in contact with the local anesthetic which determines the time course of diffusion That this in fact was the case was shown by nerve fiber counts in cross sections of the nerve (Table 14)

Proximal to the point at which the ramus profundus anterior and posterior leave the sciatic nerve, only half of the nerve fibers are activated when the distal end of the nerve is stimulated Although this portion of the anesthetized part of the nerve is hardly relevant for the time course of diffusion we*) have investigated whether the activated fibers are evenly distributed

Table 14

Number of large myelinated fibers (10–15 μ in diameter) at the site of stimulation (peroneal and tibial nerves) and in the sciatic nerve immediately distal to the branching point of ramus profundus anterior and posterior (Right (I) and left (II) nerves from the same frog)

nerve	number of fibers	
	I	II
peroneal	753 } 1309	734 } 1293
tibial	556	559
sciatic	1295	1297

*) experiments performed together with Professor K G Wingstrand

over the cross section or lie in separated bundles according to their origin. The distribution of activated fibers was studied in the following way.

A retrograde degeneration of the distally stimulated nerve fibers was produced by amputating the leg in four frogs below the knee. The tibial and peroneal nerves were cut at a level which corresponded to the site of *stimulation in the anesthesia experiments.* *The frogs were kept for three* weeks and the nerves were then fixed and stained according to the method of Marchi (ROMEIS 1948). Degenerated nerve fibers appeared as black spots in the histological sections and could easily be distinguished from other myelinated fibers. The degeneration could be followed proximally in the sciatic nerves. Sections of the nerves proximal to the site at which the stimulating electrodes were placed showed a random distribution of degenerated nerve fibers in the sciatic (Fig. 28). That only a part of the cut fibers appeared degenerated after a period of three weeks is due to the different rates of which degeneration occurs in different fibers and to the fact that only fibers in a certain state of degeneration are stained by the Marchi *method. It is however unlikely that the state of degeneration is correlated* with the distribution of the fibers in the nerve, and it seems justified to conclude *that all stimulated fibers are evenly distributed within the nerve at* the site of the postanesthetic lead.

In the literature no information about this distribution was available on frog nerves. In mammalian nerve it is still unsettled whether the fiber originating from distal branches (peroneal and tibial nerves) are evenly distributed in the sciatic nerve or lie in separate bundles. Some authors have reported an even distribution (FOERSTER 1929, SUNDERLAND and RAY 1948) *others found that the proximal part of the sciatic nerve was still organized* in bundles corresponding to the peroneal and tibial nerves (WOHLFART 1935, GUTMANN 1960).

Inside the nerve sheath diffusion is localized in the main to the interstitial space. This implies that

1) the effective area through which diffusion takes place is *considerably* smaller than the total cross section of the nerve, and 2) the path of diffusion into the nerve is longer than it would be in an homogeneous medium. If the inhomogeneity did not cause a qualitative alteration in the course of *diffusion*, this would result solely in a smaller diffusion coefficient than that for diffusion in an homogeneous medium. The figures estimated below indicate the extent to which the presence of fibers in the nerve would diminish the diffusion coefficient.

To facilitate the calculations the nerve fibers were considered as being quadratic with side D, whereas the width of the space between the fibers

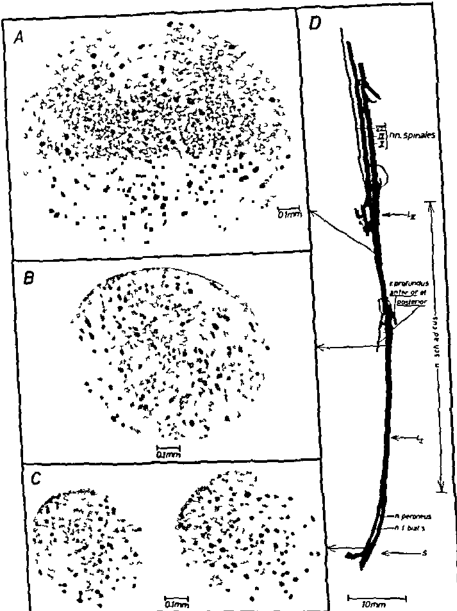


Fig 28 To show the random distribution of *nerve fibers* from the tibial and peroneal nerves in the sciatic of the frog by retrograde degeneration

A C Distribution of degenerated nerve fibers (black spots) three weeks after cutting the nerve at *S* (cf photograph of osmium stained nerve in *D*)

C Section about one mm proximal from the site of the stimulating electrodes (*S*)

B Section distal to the branching of ramus profundus anterior and posterior of the sciatic nerve

A Section proximal to the branching of *ramus* profundus anterior and posterior (fixed and stained according to Marchi s method 30 μ sections)

L_I site of pre anesthetic recording electrodes

L_{II} site of post anesthetic recording electrodes

was d. The interstitial space in frog nerve constitutes about one third and the fibers about two thirds of the total volume of the nerve (SHANES 1953 b). It follows that $D^2/(D+d)^2 \approx \frac{2}{3}$, $d/(D+d) = 0.18$, in other words only 18 per cent of the nerve's surface can be penetrated by the anesthetic. Thereby the effective diffusion coefficient was diminished to 18 per cent of the value it would have if there were free diffusion through the whole nerve's surface. With this simple model the path of diffusion was $\sqrt{2}$ times longer than with unimpeded diffusion*). Since the time of diffusion increased with the square of the diffusion path and since the effective diffusion coefficient was inversely proportional to diffusion time, the longer diffusion path caused a further halving of the effective diffusion coefficient. In summary, the presence of nerve fibers would cause an effective diffusion coefficient 9 per cent or $\frac{1}{11}$ of the coefficient if there were free diffusion into the nerve.

Hydrolysis of procaine in the nerve.

Whereas the xylocaine molecule is stable and is not split with changes in hydrogen ion concentration or by enzymes, the procaine molecule is unstable and can be hydrolyzed to diethylaminoethanol and paraaminobenzoic acid. This splitting of procaine is hastened in alkaline solution (see page 135). With the velocity constant for hydrolysis given by TERP (1919) the amount of procaine split in one hour at 20°C would be 0.1 per cent at pH 7.35 and 0.25 per cent at pH 7.8.

In the organism, especially in serum, the splitting of procaine occurs more rapidly on account of the presence of procaine esterase. It has been questioned whether the nerve tissue also contains this esterase. NORDQUIST (1952 a) believed to have demonstrated it, in disagreement with SKOU (1951). NORDQUIST measured the splitting of 0.08 mg procaine added to 67–178 mg of nerve tissue. At 20°C and pH 7.3 he found that 1 g of nerve tissue hydrolyzed about 20×10^{-6} g procaine, or 1.5–4 per cent of the procaine in an hour. The non-enzymatic splitting calculated from TERP's velocity constant would be 0.1 per cent per hour. SKOU (1951) did not find an increase in splitting when a procaine solution was added to nerve tissue. From SKOU's data (70 mg procaine, 2 hours, pH 7.35, 37°C), the reaction scheme and TERP's velocity constant, a splitting of 1.4 per cent per hour would be expected. SKOU (1951) found experimentally a splitting of 1.2 per cent per hour. The question as to whether procaine is split enzymatically in nerve must therefore be considered unsettled. From a mathematical analysis of

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*) The increase in the path of diffusion was estimated as the ratio between the length of two sides of a quadratic fiber ($2D$) and the length of a diagonal ($D \times \sqrt{2}$).

the influence of the splitting of procaine on the course of diffusion of procaine into nerve ROSENFALCK concluded, that the spontaneous hydrolysis of procaine is so small that it would not appreciably affect the time course of procaine's penetration into the nerve (see Appendix 2) The same would be the case even if the splitting should be 10 times greater than indicated above on account of the presence of procaine esterase in nerve

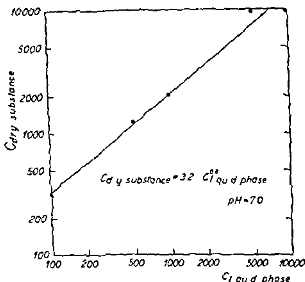


Fig 29 Distribution of procaine between the dry and liquid phases of nerve (SKOU 1954 Table 10) pH 7.0 37 C
Ordinate concentration of procaine in the dry phase (mg %)
Abscissa concentration of procaine in the liquid phase (mg %)

The binding of the anesthetic to nerve tissue

The anesthetic penetrates into the nerve via the interf...

... occurs instantaneously the expression for simple diffusion is applicable with a diffusion coefficient less than if there were no binding (CRANK 1956)

Investigations as to the binding of xylocaine to nerve tissue have not been carried out but SKOU (1954) investigated the distribution of procaine between the dry and fluid components of peripheral nerve The experiments were carried out on nerves from the cauda equina of cows at pH 7.0 and

37°C (Figure 29, from SKOU's Table 10, 1954). Procaine was bound to the dry phase according to the following relationship

$$C_{\text{dry phase}} = 3.2 \times C_{\text{watery phase}}^{0.8}$$

in other words procaine binding to the dry phase of nerve is proportional to the concentration in the watery phase to the power 0.8. According to CRANK (1956) this distribution would bring about an effective diffusion coefficient $\frac{1}{4}$ of that which obtains with free diffusion.

Conclusion: The above discussion concerns the effect of various factors *which could be imagined to invalidate the use of the mathematics of simple* diffusion to calculate a minimum concentration identical with that determined experimentally. The result was that for most of the factors there should be no principal objection to using the diffusion formula. It was, however, assumed that the inhomogeneity of the nerve did not affect the relative time course of anesthesia and that the binding of the anesthetic to the nerve tissue was firm and instantaneous. In addition the theoretical description *does not consider the effect of subminimal concentrations on the* action potential amplitude. The lack of agreement between theory and experiment may therefore be ascribed to these factors. To what extent they can account for the difference between the calculated and the experimentally determined minimum concentration remains an open question.

Chapter 6

DETERMINATION OF A VELOCITY FACTOR FOR THE TIME COURSE OF ANESTHESIA FROM DIFFUSION THEORY AND THE EXPERIMENTALLY DETERMINED MINIMUM CONCENTRATIONS

It was not possible, from the time course of anesthesia and the mathematics of free diffusion to determine both a minimum concentration of the anesthetic identical with that measured directly and a diffusion coefficient, identical in experiments with different concentrations (Chapter 5) The question remains, whether an empirical expression can be found which allows prediction of the time course of anesthesia at different concentrations and on nerves with different diameters The effect of a given local anesthetic on the single nerve fiber is adequately described by the minimum concentration required to block the fiber In whole nerve there is an additional factor in that it takes time for anesthesia to develop The time course of anesthesia is not a simple function of either the outer concentration or the minimum concentration In the following an attempt is made to characterize the rapidity with which the anesthetic penetrates into the nerve by introducing a velocity factor This would allow prediction of the time course of anesthesia with different concentrations and for nerves of different diameter It would facilitate the comparison of the effect of a given anesthetic under different conditions If, for example changes are introduced in the local anesthetic or in the tissue which might change its penetration into the nerve, this would appear in different velocity factors Furthermore – aside from the minimum concentration – a velocity factor might be used in evaluating the effectiveness of different anesthetics on whole nerves

Since the anesthetic penetrates into the nerve by diffusion, it seems near at hand to test the possibility of predicting the time course of anesthesia by means of the differential equation for free diffusion in a cylinder and the experimentally determined minimum concentration D in equation (2) page 85 would in this case be considered a common *velocity factor* for the time course of anesthesia at various concentrations of the local anesthetic

37°C (Figure 29, from SKOU's Table 10, 1954) Procaine was bound to the dry phase according to the following relationship

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The determination of a velocity factor for xylocaine in nerves with sheath.

To test the possibility of introducing a common velocity factor D for experiments with different concentrations of the anesthetic the factor D was determined for each experiment in the following way Among the theoretically calculated time courses of anesthesia (Fig 26 A) the one is selected which has the same ratio between the base concentration of the anesthetic and the minimum concentration as the experimental curve The velocity factor is then determined as that value of D for which the theoretical curve is in closest agreement with the experimental curve Examples of agreement between the experimental and the theoretical curves are shown in Figure 30 A–C In 30 of 46 experiments with xylocaine the difference between the experimental and the theoretical amplitude values did not exceed 6 per cent in the amplitude range 1–0 2 (initial amplitude = 1), and in the amplitude range 0 2–0 the maximum amplitude deviation was 0 06 amplitude units (Table 15) When the deviation was expressed in time there was a significant difference between the experimental and theoretical courses only in the beginning of the course of anesthesia

In eight experiments (example in Figure 30 D) the maximal difference was 9 per cent between the experimental and theoretical amplitude in the range 1–0 2 When the deviation for these experiments was expressed in time the deviation in the beginning of the course of anesthesia at an amplitude of 0 75 was twice as great as the deviation in the 30 experiments described

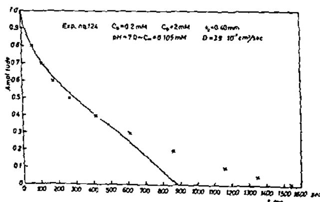
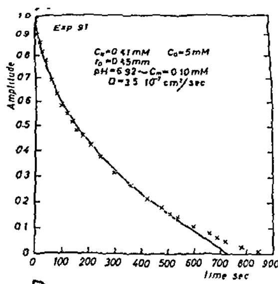
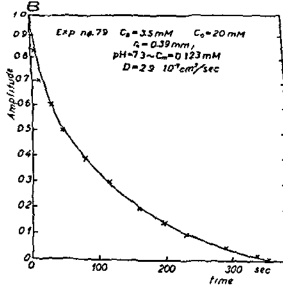
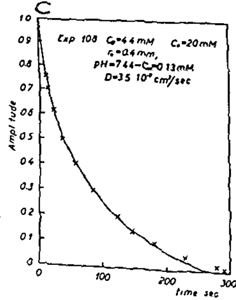


Fig 31 Example of deviation of the experimental from the calculated (solid line) time course of xylocaine anesthesia (nerve with sheath) The theoretical time course was calculated from diffusion theory and the experimental minimum concentration Ordinate action potential amplitude in units of the amplitude in Ringer's Abscissa time in seconds after application of the anesthetic







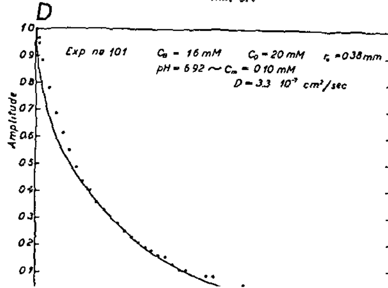


Fig 30 Examples of the agreement (A, B, C) between experimental and calculated (solid lines) time course of xylocaine anesthesia (nerves with sheath)

Ordinate action potential amplitude in units of the amplitude in Ringer s

Abscissa time in seconds *after* application of the anesthetic (Note different time scales)

The *theoretical time* course was calculated from diffusion theory and the experimental minimum concentrations

D is an example of experiments in which the calculated decrease in action potential amplitude initially was slightly larger than the experimental

above, whereas at the lower amplitude levels there was the same agreement as in the 30 experiments described above

In the remaining eight experiments there was poor agreement between the experimental and the theoretical course In five experiments, with base concentrations less than two times the minimum concentration, the last portion of the experimental course of anesthesia was clearly slower than the theoretical (Figure 31) In three experiments there was a marked deviation in the beginning

The mean value of the velocity factor for xylocaine in the 30 experiments with agreement between the experimental and theoretical course of anesthesia was $2.9 \times 10^{-7} \pm 0.2 \times 10^{-7}$ cm²/sec and for all 16 experiments $3.0 \times 10^{-7} \pm 0.15 \times 10^{-7}$ cm² sec (S D = 35 per cent)

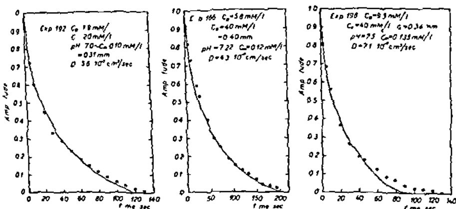


Fig 32 Examples of the agreement bet een expe ... tim

Ord ▪

Abscissa time in seconds after application of the anesthetic (Note different time scales)

The theoretical time course was calculated from diffusion theory and the experimental minimum concentration

Determination of the velocity factor for xylocaine in nerves without sheath. In experiments on nerves without sheath agreement was found between the experimental and the calculated course of anesthesia for action potential amplitudes above 10–15 per cent of the initial value when the experimentally found minimum concentrations was used, when the amplitude was below 10–15 per cent the experimental time course was in general slightly slower than the theoretical (Figure 32)

In 14 of the 16 experiments carried out at a base concentration greater

Table 15

Differences between the experimental and calculated time courses of anesthesia in 16 experiments with xylocaine. The theoretical course calculated from diffusion theory and the experimental minimum concentration

Difference in per cent between the experimental and calculated time to various levels of action potential amplitude

number of experiments	amplitude level	0.75	0.35	0.10	0
30	difference	30	0	0	15
	S.D.	27	5	7	19
8	difference	65	— 4	— 1	10
	S.D.	24	14	10	18
3	difference	110–140	0–12	— 6–0	0–25
5	difference	0–8	0–20	15–135	50–200

Difference between experimental and calculated action potential amplitude in various amplitude ranges (in units of the initial amplitude)

number of experiments	amplitude range	1–0.5	0.5–0.2	0.2–0
30	difference	0.015	0.023	0.030
	S.D.	0.020	0.015	0.010
8	difference	0.085	0.030	0.034
	S.D.	0.020	0.015	0.020
3	difference	0.01–0.14	0.01–0.04	0.02–0.03
5	difference	0.01–0.03	0.02–0.13	0.06–0.20

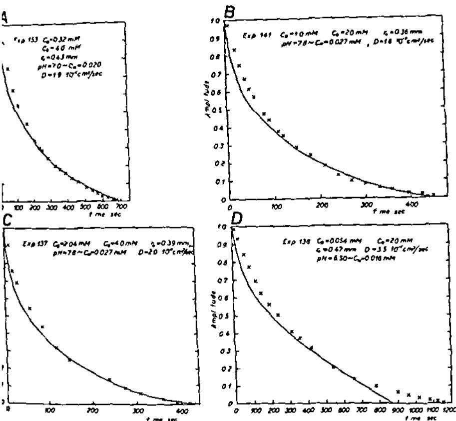


Fig 33 Comparison of the experimental and calculated (solid lines) time course of procaine anesthesia in nerves with sheath

Ordinate action potential amplitude in units of the amplitude in Ringer s

Abscissa time in seconds after application of the anesthetic (Note different time scales)

The theoretical time course was calculated from diffusion theory and the experimental minimum concentrations A B, C are examples of agreement between experiment and theory in the last portion of the course of anesthesia D is an example of a disagreement between theory and experiment

cylinder, the experimentally determined minimum concentrations at the pH of the solution and the experimental time courses of anesthesia, a common velocity factor D for anesthesia could be calculated which allowed prediction of a major portion of the time course of anesthesia at different concentrations

With xylocaine the time course could be predicted until the action potential amplitude was reduced to 15-20 per cent of its initial value, on nerves with and without sheath and with concentrations 3 to 90 times the minimum

than the minimum concentration the deviation in the amplitude range 1–0 5 did not exceed 0 06 amplitude units or 8 per cent In the amplitude range 0 5–0 2 the maximal deviation was 0 03 amplitude units or 7 per cent In the amplitude range 0 2–0 the maximum deviation did not exceed 0 021 amplitude units In two experiments at xylocaine base concentrations 2-3 times the minimum concentration (nos 125 and 189) there was poor agreement between the theoretical and experimental curves

The mean value of the velocity factor for xylocaine in the 11 experiments on nerves without sheath with agreement between theory and experiment was $8\ 9 \times 10^{-7} \pm 0\ 8 \times 10^{-7}$ cm²/sec (S D = 31 per cent)

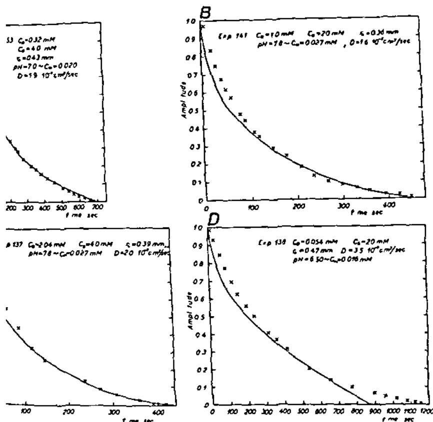
Determination of the velocity factor for procaine in nerves with sheath.

In 13 of 16 experiments with procaine there was agreement between the experimental and theoretical time course *in the range* 0 35–0 amplitude units with a maximum deviation of 0 03 amplitude units (Fig 33 A, B, C) In the initial course of the anesthesia, i e the amplitude range 1–0 35 the *experimental course in these 13 experiments was slower than the theoretical* The maximum amplitude deviation in this range averaged 0 13 amplitude units corresponding to 25 per cent Expressed as deviation in time, the experimental time interval required for a reduction in action potential amplitude to 0 75 amplitude units was on the average twice as long as the theoretical (S D 31 per cent), in the amplitude range 0 35 to total block there were no significant differences between theoretical and experimental curves

In three experiments with base concentrations lower than 3 5 times the minimum concentration (Fig 33 D) the last portion of the experimental course progressed considerably slower than the theoretical Thus, the agreement *between theory and experimental findings was not as close in the case of procaine as in the case of xylocaine*

The velocity factor determined for the 13 experiments with procaine where agreement with theory was obtained *in the later part of the anesthesia* averaged $2\ 1 \times 10^{-7} \pm 0\ 1 \times 10^{-7}$ cm²/sec (S D = 19 per cent)

Discussion. In the previous chapter it was shown that the model representing free diffusion of the anesthetic substance was at variance with the experimental findings, in that the minimum concentrations calculated from the model differed from those found experimentally Nor were the calculated diffusion coefficients the same at all concentrations of the anesthetic On the other hand from the differential equation describing free diffusion in a



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CHAPTER 7

THE RESTITUTION OF THE NERVE ACTION POTENTIAL AFTER ANESTHESIA

This chapter deals with the time course of restitution of the nerve action potential after local anesthesia with various concentrations of xylocaine and procaine base Furthermore an attempt will be made to determine the extent to which the course of restitution can be described in terms of diffusion of the anesthetic out of the nerve

When the nerve is just fully blocked the anesthetic is distributed in the nerve as illustrated in Fig 34 a The most central fiber has just been exposed to a concentration equal to the minimum concentration Maintaining the anesthetic around the nerve diffusion will continue into the nerve until the concentration in the nerve is the same as that outside it (Fig 34 b) Replacing the bathing solution with Ringer s solution the anesthetic diffuses out of the nerve When the concentration in the peripheral portions of the nerve falls just below the minimum concentration (Fig 34 c) the first signs of activity appears Investigations along these lines should allow a mathematical analysis of the time course of diffusion There is however the disadvantage that there is no criterion to indicate when the nerve is fully saturated with the anesthetic though the time required for full saturation could be estimated theoretically

Instead of waiting for full saturation anesthesia was arrested when the most central fibers were blocked (Fig 34 a) This time can be measured exactly since it coincides with the time at which the action potential disappears This has the consequence however that further diffusion is complicated in that the anesthetic diffuses both into and out of the nerve At a given time after substitution of the anesthetic with Ringer s and while there is still full anesthesia the conditions are approximately as shown in Fig 34 d (see also Fig 15) At the first sign of activity the distribution of the anesthetic within the nerve should be as shown in Fig 34 e

An example of the restitution of the nerve action potential amplitude the xylocaine solution having been replaced by Ringer s solution when the

concentration The experimental blocking time was longer than that de mined theoretically With procaine the range of amplitudes in which velocity factor could be used was narrower than for xylocaine in that the later part of the time course of anesthesia could be predicted, i e range from 35 per cent of the amplitude in Ringer's to full block, for centrations of 3 5–80 times the minimum concentration

It appears from these findings that the empirical expression derived f diffusion theory combined with the experimentally determined values of minimum concentration allowed prediction of the major part of the course of local anesthesia with xylocaine in different concentrations nerves with and without sheaths The faster action of the local anesth in nerves without sheaths appeared as an increase in the velocity factor

As illustrated by the findings with xylocaine and procaine, predictio the time course of local anesthesia with different anesthetics meets difficulty that the agreement between experimental and calculated co may occur at different degrees of anesthesia Therefore, under these circ stances the velocity factor does not characterize differences in the ac of different local anesthetics

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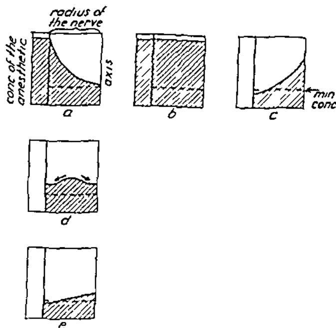


Fig 34 Schematic illustration of the distribution of the anesthetic within the nerve at various times after its application and removal

a the distribution at the time when the nerve is just blocked by the anesthetic

b the nerve is fully saturated with the outer concentration of anesthetic

c the saturated nerve is placed in Ringer s and diffusion takes place out of the nerve When the concentration at the nerve surface is below the minimum concentration the nerve begins to respond again

d the anesthetic solution is substituted with Ringer s when the nerve is just fully anesthetized (cf a) Initially the anesthetic diffuses both into and out of the nerve

e later, when the peripheral concentration is below the minimum concentration the first evidence of nerve activity is obtained

nerve was just fully blocked, is illustrated in Fig 35 (see also Fig 16) About 25 minutes elapsed before the nerve developed an action potential After the action potential reappered it increased in amplitude to about 5 per cent of the normal value within 10 15 minutes During the next 20 minutes it increased nearly rectilinearly to about 80 per cent of normal amplitude, to approach 100 per cent within the next 30 minutes

The onset of restitution was defined as the time interval after replacement of the anesthetic with Ringer s until the amplitude was restored to 1 per cent of the normal amplitude (I_{001}) The time interval until the amplitude attained 100 per cent was difficult to determine because of the diminished slope late in the course of restitution Therefore I chose the time interval at which the amplitude reached 50 per cent of normal amplitude (T_{05}) as a measure of the duration of restitution To compensate for differences in nerve thickness these time intervals were divided by the square of the radius

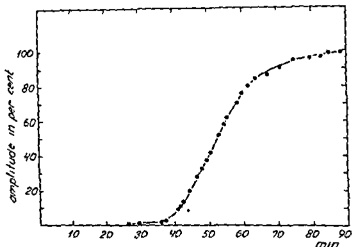


Fig 35 Restitution of the nerve action potential amplitude after anesthesia with xylocaine (C_B 3 7 mM, nerve radius 0 37 mm, 22 C, nerve without sheath)
Ordinate amplitude in per cent of the amplitude in Ringer's before anesthesia
Abscissa time in minutes after substitution of xylocaine with Ringer s

Restitution of the action potential after anesthesia of nerves with sheath.

In 17 experiments the restitution of the nerve action potential was studied after anesthesia with various concentrations of xylocaine and procaine

After the blocked nerve was replaced in Ringer's solution it took 10–90 minutes for the action potential to return to 1 per cent of its normal value except when the concentration of anesthetic was very close to the minimum concentration The time required for the action potential to regain 50 per cent of its normal amplitude was about twice as long as the time to 50 per cent anesthesia when the concentrations were low (experiments 139 and 156), with the largest concentrations used (experiments 137 and 162) restitution took 150 230 times as long as anesthesia These differences can be explained in part by the course of diffusion (cf p 150)

The correlation between restitution times (to onset and to 50 per cent restitution) and concentration of xylocaine and procaine base was obtained in experiments with different concentrations of the anesthetics (Tables 16, 17, Fig 36 a, b) In the xylocaine experiments the base concentrations of the anesthetic was varied between 0 15 mM and 13 7 mM In procaine a group of 5 experiments were performed with concentrations of 1 to 1 1 mM and allowed a calculation of the standard deviation of the time to the onset of restitution ($T_{0\,01}$ r_0^2) This was 10 per cent

Table 16

Restitution of action potential amplitude after anesthesia with different xylocaine concentrations and pH (nerves with sheath)

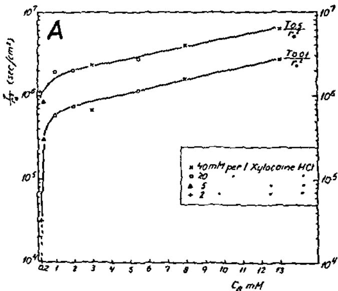
experiment no	hydrochloride concentration (C_0) mM	pH	base concentration (C_B) mM	temperature °C	nerve radius (r_0) mm	$T_{0.01}$ minutes	$\frac{T_{0.01}}{r_0^2}$ sec/cm²	$T_{0.5}$ minutes	$\frac{T_{0.5}}{r_0^2}$ sec/cm²
156	2	6.98	0.15	22.7	0.39	0.75	2.90×10^4	29.0	1.12×10^6
136	5	6.84	0.33	23.5	0.15	10.5	3.05×10^5	29.3	8.56×10^5
157	20	6.70	1.00	23.1	0.10	16.0	5.85×10^5	54.0	1.97×10^6
151	*20*	*7.02*	*2.00*	*22.7*	*0.10*	*19.75*	7.32×10^5	*54.0*	2.0×10^6
159	40	6.90	3.00	22.8	0.44	22.0	6.74×10^5	77.5	2.37×10^6
161	20	7.57	5.5	23.0	0.46	40.75	1.15×10^6	99.3	2.8×10^6
160	40	7.40	8.1	22.2	0.14	52.0	1.61×10^6	131.0	4.07×10^6
162	10	7.72	13.7	22.1	0.13	88.0	2.86×10^6	191.0	6.3×10^6

Table 17

Restitution of action potential amplitude after anesthesia with different procaine concentrations and *pH (nerves with sheath)*

experiment no	hydrochloride concentration (C_0) mM	pH	base concentration (C_B) mM	temperature °C	nerve radius (r_0) mm	$T_{0.01}$ minutes	$\frac{T_{0.01}}{r_0^2}$ sec/cm²	$T_{0.5}$ minutes	$\frac{T_{0.5}}{r_0^2}$ sec/cm²
139	20	6.00	0.017	22.2	0.38	1.8	7.80×10^4	31.0	1.44×10^6
138	*20*	6.50	0.054	*23.3*	0.47	*6.2*	1.72×10^5	31.0	8.58×10^5
135	*40*	7.00	*0.33*	*21.5*	0.45	*17.7*	5.20×10^5	42.5	$1.2[illegible] \times 10^6$
140	20	7.80	1.0	25.6	0.35	33.0	1.64×10^6	65.0	3.22×10^6
146	20	7.80	1.0	25.3	0.43	43.5	1.44×10^6	90.0	2.9×10^6
150	20	7.80	1.0	23.9	0.45	*63.0*	1.83×10^6	*113.5*	3.3×10^6
149	20	7.80	1.0	21.5	0.41	50.5	1.80×10^6	87.0	3.1×10^6
147	40	7.55	1.1	25.5	0.40	50.0	1.84×10^6	88.0	3.24×10^6
137	10	7.80	2.0	23.2	0.39	75.0	2.90×10^6	176.0	6.82×10^6

$T_{0.01}$ and $T_{0.5}$ are the times from replacement of the anesthetic by Ringer's till the action potential amplitude was 1 per cent and 50 per cent of the amplitude in Ringer's before anesthesia



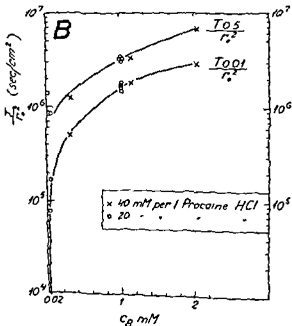


Fig 36 Restitution time for the nerve action potential amplitude after anesthesia with different xylocaine (A) and procaine (B) base concentrations (C_B) Nerves with sheath. (Temperature range 22 1–25 6 C)
Ordinate [illegible] by division by the square of the radius (r_0) (sec/cm²) (log scale)
Abscissa base concentration of the anesthetic (mM)
The symbols indicate hydrochloride concentrations.

The restitution time varied systematically with base concentration independent of the hydrochloride concentration and the pH at which it was obtained (Fig 36) As a function of acid concentration there was no systematic variation in restitution time In Figure 36 consideration is not taken of the various pH values at which the experiments were carried out Correcting for these and expressing the base concentrations in units of the minimum concentration the restitution time increased systematically with the concentration

Another way of determining the minimum concentration is to reduce the outer concentration of the anesthetic to the extent that the first sign of activity appeared at the earliest time after substitution of the anesthetic by Ringer s solution

In Fig 36 there are only a few experiments with base concentrations close to the minimum concentration The minimum concentrations obtained by extrapolation were for xylocaine and procaine in agreement with those found experimentally (cf p 60)

That the restitution time in fact is very short (a few minutes) when the concentration of the anesthetic was close to the minimum concentration was also apparent in the experiments in which the minimum concentration was determined directly The nerves which were blocked with minimum concentration (4 nerves with a xylocaine base concentration of 0.01 mM pH 6.0 6 nerves with a procaine base concentration of 0.013 mM at pH 6.12 and 4 nerves with 0.021 mM at pH 7.08) gave a measurable action potential 1 2 minutes after the nerve was transferred from the anesthetic solution into Ringer s solution That the action potential did not appear immediately might be due to binding of the anesthetic to the nerve tissue

Restitution of the action potential after anesthesia of nerves without sheath

Fig 37 shows the values of $T_{0.01}/r_0$° and $T_{0.5}/r_0$° from 10 experiments on nerves without sheath as a function of the concentration of xylocaine base used for anesthesia The solid lines refer to experiments on nerves with *intact sheaths (see Fig 36 a)*

The restitution time was the same in nerves without as in those with sheath The same result was obtained by Truant and Lanzoni (1952) The time course of restitution was not determined by diffusion alone but influenced by binding of the anesthetic to the nerve tissue (cf Appendix 3) That the removal of the nerve sheath did not affect the restitution times indicates that the effect of binding is greater than the delaying effect of the sheath on the diffusion of the anesthetic out of the nerve

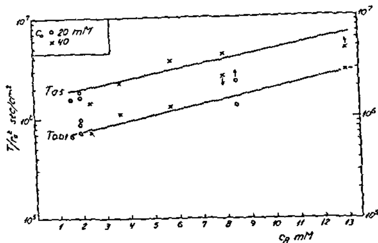


Fig 37 The restitution of action potential amplitude after anesthesia with various *xylocaine* base concentrations (C_B) in nerves without sheath (temperature range 20.1–25.3° C)

Ordinate time after substitution of xylocaine by Ringer's until amplitude *regains* 1 per cent ($T_{0.01}$) and 50 per cent ($T_{0.5}$) of normal The time is compensated for variations in nerve thickness by division by the square of the radius (r_0) (sec/cm²) (log scale)

Abscissa xylocaine base concentration (mM)

The solid lines indicate the average restitution times for nerves with sheath (*cf* Fig 36) The symbols refer to different hydrochloride concentrations

Comparison between restitution times after anesthesia with xylocaine and procaine.

In Table 18 the restitution times to 1 and 50 per cent amplitude are compared for 2 and 4 mM xylocaine and procaine base The nerve impulse was restored about three times faster after xylocaine than after procaine anesthesia The difference was somewhat more pronounced for the time to 1 than to 50 per cent amplitude restitution

The restitution times may also be compared after anesthesia with the same concentrations of xylocaine and procaine *hydrochloride at the same pH* Considering for example 10 mM xylocaine and procaine hydrochloride at pH 7.3 this corresponds to base concentrations of 6.6 and 0.65 mM respectively (Table 19) There was only a slight difference in the restitution times in that the procaine-anesthetized nerve was restored about one and a half times faster than the xylocaine anesthetized

8*

Table 18

Restitution after anesthesia with xylocaine and procaine with the same base concentration

base concentration (C_B) mM		$\frac{T_{0\,01}}{r_0^2}$ sec/cm²	$\frac{T_{0\,01}\text{ procaine}}{T_{0\,01}\text{ xylocaine}}$	$\frac{T_{0\,5}}{r_0^2}$ sec/cm²	$\frac{T_{0\,5}\text{ procaine}}{T_{0\,5}\text{ xylocaine}}$
2	procaine	$2\ 9 \times 10^6$	3 8	$6\ 8 \times 10^6$	3 4
	xylocaine	$7\ 6 \times 10^5$		$2\ 0 \times 10^6$	
1	procaine	$1\ 6 \times 10^6$	2 8	$3\ 2 \times 10^6$	2 0
	xylocaine	$5\ 8 \times 10^5$		$1\ 6 \times 10^6$ *)	

*) interpolated value from the curve Fig 36A

Table 19

Restitution after anesthesia with xylocaine and procaine with the same hydrochloride concentration

	base concentration at pH 7 3 (C_B) mM	$\frac{T_{0\,01}}{r_0^2}$ sec/cm²	$\frac{T_{0\,01}\text{ procaine}}{T_{0\,01}\text{ xylocaine}}$	$\frac{T_{0\,5}}{r_0^2}$ sec/cm²	$\frac{T_{0\,5}\text{ procaine}}{T_{0\,5}\text{ xylocaine}}$
procaine HCl 40 mM	0 65	$1\ 0 \times 10^6$	0 77	$2\ 1 \times 10^6$	0 64
xylocaine HCl 40 mM	6 6	$1\ 3 \times 10^6$		$3\ 3 \times 10^6$	

The restitution times have been interpolated from the curves of Fig 36

Discussion. Clinical studies have indicated that there is a correlation between the concentration of the anesthetic and the restitution time after anesthesia With the wheal test and corneal reflex in rabbits as indicators there was a logarithmic relation between the duration of anesthesia and the

concentration of the anesthetic (SINHA 1939 and MIESCHER 1941) GOLDBERG (1941) and BJORN (1946) found that the larger the concentration of anesthetic the more rapid the onset of anesthesia and the longer its duration

I have found the restitution time both for xylocaine and for procaine to increase with the base concentration as has been shown previously by GARDNER, SEMB and GRAHAM (1931) The orderliness of this relationship is a further indication that it is the free base which is the active anesthetic component

BENNETT et al (1942) and TRUANT and LANZONI (1952) observed, as I have found that the restitution time was considerably longer than the time to nerve block They consider that this cannot be explained by the course of diffusion but assume that the anesthetic is bound to nerve tissue thus delaying diffusion out of the nerve

During the development of the anesthesia there is usually a large concentration gradient between the outer solution and the interior of the nerve so that diffusion progresses rapidly During restitution the gradient rapidly diminishes between the interior of the nerve and the surroundings and diffusion progresses more slowly If the anesthetic is removed just after block is achieved diffusion of the anesthetic continues from the periphery to the centre of the nerve and thereby the diffusion time out of the nerve is further increased

In addition to the concentration gradient the diffusion of the anesthetic out of the nerve depends on its radius, the velocity factor and the minimum concentration The greater the minimum concentration of the anesthetic the more rapidly is the fall in the concentration peripherally below the minimum concentration and thereby the peripheral fibers regain their ability to conduct impulses That the experimental time course of restitution was qualitatively determined by these factors may be seen by comparing the restitution times after anesthesia with the same amounts of xylocaine and of procaine base Whereas the size of the velocity factor is of the same order, xylocaine base had a higher minimum concentration than procaine base, and the nerve was restituted fastest after xylocaine anesthesia (Table 18)

The question is however whether minimum concentration, base concentration nerve radius and velocity factor which in the main can account for the time course of the reduction in amplitude (Chapter 6), can account quantitatively for the experimentally found time course of restitution

ROSENFALCK found that restitution took considerably longer than to be expected from simple diffusion theory (Appendix 3, Fig 47) Thus the time from substitution of the solution of the local anesthetic with Ringer's until the action potential regained 1 per cent of the initial amplitude was 20–40

times longer than to be expected if the time course of restitution were determined solely by diffusion of the anesthetic, using the values for velocity factor and minimum concentration found in chapter 6 and 4. Considering the times until the amplitude returned to 50 per cent of the initial amplitude in Ringer's, *the experimentally found times were 2-3 times longer than* those theoretically to be expected. The later onset of restitution than theoretically expected might be explained as suggested by BENNETI et al (1942) and TRUANT and LANZONI (1952), by binding of the anesthetic to nerve tissue, delaying diffusion of the anesthetic out of the nerve.

Considering the restitution times after anesthesia with the same amounts of xylocaine and procaine *hydrochloride* at the same pH, it was found that *the nerve was restituted slightly more rapidly after procaine anesthesia*. The cause for this must be, that the concentration of procaine base was less than of xylocaine base, the restitution time depending on the base concentration.

CHAPTER 8

THE EFFECT OF LOCAL ANESTHETICS ON THE CONDUCTION VELOCITY OF THE NERVE ACTION POTENTIAL

KATO (1926), TAMURA (1928) and TOMAN, WOODBURY and WOODBURY (1947) demonstrated that the conduction velocity decreases in that portion of a nerve subjected to cocaine and procaine as the anesthesia progresses I have confirmed these findings for procaine and have found a similar relationship for xylocaine (Figs 38 39)

There were two possible causes for this decrease in conduction velocity

1) the conduction velocity was diminished in all fibers in contact with concentrations of the local anesthetic below the minimum concentration

2) the most rapid fibers were affected first and were therefore blocked before the slow fibers

To decide between these possibilities the conduction velocity was determined in the postanesthetic portion of the nerve A third pair of recording electrodes was placed on the nerve proximal to those from which the postanesthetic recording was taken If the conduction velocity were diminished on account of a selective block of the fast fibers, one would expect to find a reduction in conduction velocity both in the anesthetized and the post anesthetic section of the nerve If the conduction velocity were diminished in fibers which still conducted but were affected by subminimal concentrations of the anesthetic these fibers would again conduct with normal velocity in the postanesthetic section of the nerve

The result of three experiments with 0 25 mM xylocaine base was that the conduction velocity measured in the postanesthetic section of the nerve was the same as the conduction velocity measured in Ringer s, indicating that the local anesthetic in subminimal concentrations reduces the conduction velocity of the single fiber Similar results were obtained with ether (BOHLTTAL and FROLICH 1901) the conduction velocity being diminished in the anesthetized section of nerve and normal distal and proximal to the affected nerve section

times longer than to be expected if the time course of restitution were determined solely by diffusion of the anesthetic, using the values for velocity factor and minimum concentration found in chapter 6 and 4. Considering *the times until the amplitude returned to 50 per cent of the initial amplitude* in Ringer's, the experimentally found times were 2–3 times longer than those theoretically to be expected. The later onset of restitution than theoretically expected might be explained as suggested by BENNETT et al. (1912) and TRUANT and LANZONI (1952), *by binding of the anesthetic to nerve tissue*, delaying diffusion of the anesthetic out of the nerve.

Considering the restitution times after anesthesia with the same amounts of xylocaine and procaine *hydrochloride* at the same pH, it was found that the nerve was restituted slightly more rapidly after procaine anesthesia. The cause for this must be, that the concentration of procaine base was less than of xylocaine base, the restitution time depending on the base concentration.

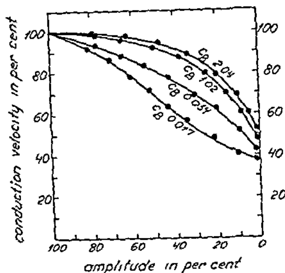


Fig 39 Diminution in conduction velocity as a function of progressing anesthesia for different concentrations of procaine base C_B in mM indicated on the curves (22°C)
Ordinate conduction velocity in per cent of the value in Ringer's
Abscissa nerve action potential amplitude in per cent of the value in Ringer's

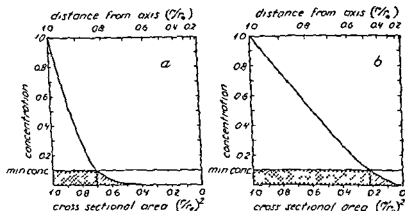


Fig 40 Diagram to illustrate the effect of subminimal concentrations on nerve conduction velocity at different times after application of the anesthetic to the nerve (Constructed from Fig 25)

Ordinate concentration of the anesthetic in units of the outer concentration
Upper abscissa distance (r) from the axis in units of the nerve radius (r_0)
Lower abscissa the area within the distance (r) from the axis in units of the cross sectional area of the nerve

The cross hatched area indicates the blocked portion of the nerve, the hatched area indicates the fraction of the nerve affected by subminimal concentrations

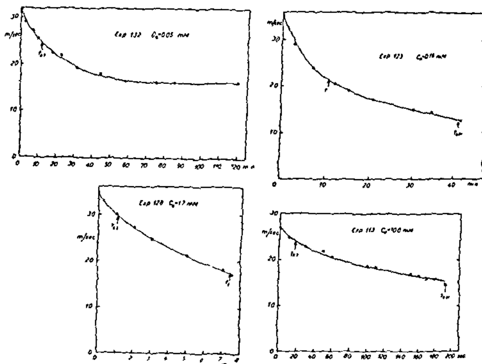


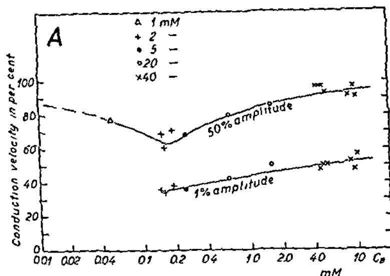
Fig 38. Examples of the diminution in conduction velocity with time during anesthesia
Ordinate conduction velocity in m/sec
Abscissa time after application of xylocaine (Note different time scales)
C_B indicates the xylocain base concentration, $t_{0\,5}$ and $t_{0\,01}$ indicate the times at which the amplitude was reduced to 50 and 1 per cent of the pre-anesthetic value (Corrected to temperature 22° C)

Experiments are described below in which the diminution in action potential conduction velocity was determined as related to

1) the degree of anesthesia of the nerve,
2) the base concentration of the anesthetic and
3) anesthesia with xylocaine and procaine.

The diminution in conduction velocity during the development of anesthesia.

In Table 20 are given the conduction velocities in the anesthetized section of the nerve measured when the action potential amplitude amounted to 50 and 1 per cent of its initial value The velocity decreased most rapidly immediately after application of the anesthetic and more slowly thereafter



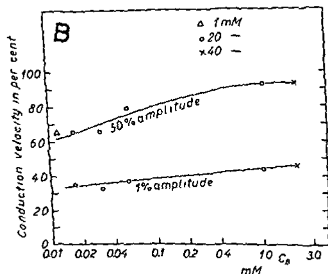


Fig 41 Correlation between conduction velocity and xylocaine (*A*) and procaine (*B*) base concentration at 50 and 99 per cent block (22°C).
Ordinate conduction velocity in per cent of the value in Ringer's
Abscissa base concentration of the anesthetic in mM (log scale)
The symbols indicate hydrochloride concentrations

Table 20

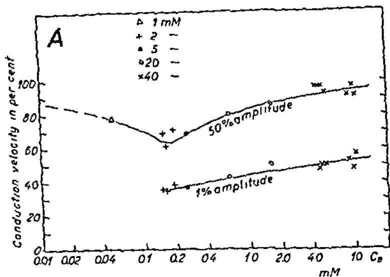
Conduction velocity during anesthesia with different xylocaine and procaine base concentrations at times to half action potential amplitude ($t_{0.5}$) and to block ($t_{0.01}$) 22°C

experiment no	anesthetic	hydrochloride concentration (C_0) mM	pH	minimum concentration *) (C_m) mM	base concentration (C_B) mM	C_B/C_m	conduction velocity in per cent of velocity in Ringer's	
							$t_{0.5}$	$t_{0.01}$
132	xylocaine	1	6 70	0 09	0 05	0 55	75 8	—
115	—	2	6 88	0 10	0 15	1 5	68 0	35 5
123	—	2	6 90	0 10	0 16	1 6	61 0	31 3
121	—	2	7 00	0 11	0 19	1 7	70 6	37 6
188	—	5	6 72	0 09	0 25	2 8	68 0	35 8
107	—	20	6 50	0 07	0 63	9 0	79 0	42 0
128	—	20	6 99	0 11	1 80	17 2	86 0	50 4
130	—	40	7 09	0 11	4 47	40 6	96 0	47 6
110	—	40	7 12	0 11	4 78	43 5	96 0	50 0
111	—	40	7 16	0 12	5 13	42 8	92 5	50 0
112	—	40	7 41	0 13	8 71	67 3	91 0	52 5
114	—	40	7 49	0 14	9 6	68 6	96 3	16 8
113	—	40	7 52	0 14	10 0	72 1	88 8	56 6
170	procaine	1	7 18	0 022	0 012	0 55	65 0	—
139	—	20	6 00	0 010	0 017	1 7	65 8	31 9
151	—	20	6 20	0 014	0 030	2 1	65 5	32 2
138	—	20	6 50	0 016	0 054	3 4	79 0	37 4
141	—	20	7 80	0 025	1 0	40 8	92 0	13 2
137	—	40	7 80	0 025	2 0	81 6	93 0	16 0

*) minimum concentrations from Fig 18

(Fig 38) The relationship between action potential amplitude and conduction velocity is shown in Fig 39 With decreasing amplitude (increasing degrees of nerve anesthesia) the conduction velocity decreased

This progression of events can be explained as the effect of the increasing effect of subminimal concentrations as the anesthetic penetrates into the nerve The higher the concentration surrounding the active fibers the greater will be the reduction in conduction velocity The distribution of concentrations in the nerve shortly after the application of the anesthetic is shown in Figure 40 a the majority of the active fibers are subjected to



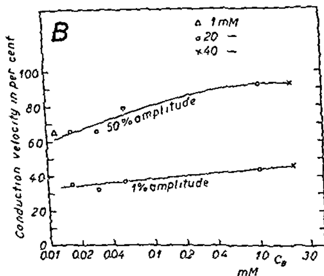
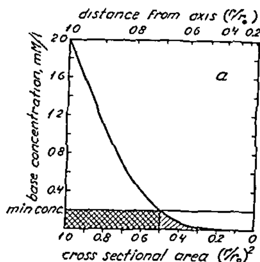


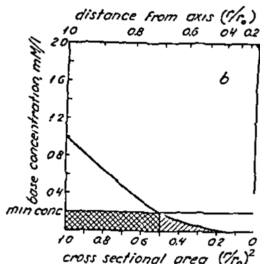
Fig 41 Correlation between conduction velocity and xylocaine (*A*) and procaine (*B*) base concentration at 50 and 93 per cent block (22°C)

Ordinate conduction velocity in per cent of the value in Ringer's

Abscissa base concentration of the anesthetic in mM (log scale)

The symbols indicate hydrochloride concentrations





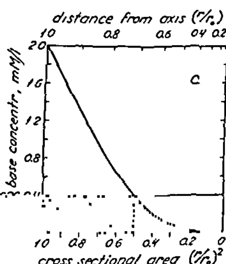


Fig 42 Diagram to illustrate the effect of subminimal concentrations of the anesthetic on nerve conduction velocity when half of the fibers are blocked (Constructed from Fig 25)

	outer base concentration in mM	minimum base concentration in mM
a	2 0	0 2
b	1 0	0 2
c	2 0	0 4

Ordinate — base concentration of the anesthetic in mM
Upper abscissa — *distance (r) from axis in units of the nerve radius (r_0)*
Lower abscissa — the area within the distance (r) from the axis in units of the cross sectional area of the nerve

The cross hatched area indicates the blocked portion of the nerve, the hatched area indicates the fraction of the nerve affected by subminimal concentrations

such low concentrations of the anesthetic that they conduct with normal velocity. A small number of fibers affected by larger but still subminimal concentrations conduct with reduced velocity. Figure 40 b shows the situation when the anesthetic has penetrated further into the nerve, when all active fibers are affected by subminimal concentrations. The overall conduction velocity at this time is therefore less than previously.

The diminution in conduction velocity with different concentrations of the anesthetic.

In 19 experiments the nerves were anesthetized with various concentrations of xylocaine and procaine base (Figs. 41 a, b, Table 20).

At the same degree of anesthesia (50 and 1 per cent amplitude) the conduction velocity diminished with decreasing base concentration for concentrations above the minimum concentration. The effect of a given base concentration was independent of the hydrochloride concentration of the anesthetic. The conduction velocity was lowest at concentrations near the minimum concentration (Chapter 4 page 60). With a concentration less than the minimum concentration (xylocaine base 0.05 mM) the diminution in conduction velocity was 24 per cent when the action potential amplitude had reached half its initial value as compared with 37 per cent with concentrations just above the minimum concentration. With high concentrations (Fig. 42 a) the portion of the cross sectional area of the nerve affected by subminimal concentrations, which reduce conduction velocity, constitutes a smaller portion of the active area than at low concentrations (Fig. 42 b). This is due to the fact that the concentration gradient in the nerve is greater when the concentration is high. The greatest diminution in conduction velocity is obtained when the active fibers are affected by the highest subminimal concentration. Therefore, for concentrations below the minimum concentration the diminution in conduction velocity is the less the lower the concentration.

Experiments with subminimal concentrations of the local anesthetics when the action potential is reduced in amplitude without being blocked allow to evaluate the correlation between the concentration of the anesthetic and the reduction in conduction velocity. For single nerve fibers TASAKI (1953) has described such a relationship for urethane. Similar findings were made with xylocaine. With 0.05 mM xylocaine base the action potential amplitude levelled off to 27 per cent of its initial value in the course of about one hour. At the same time the conduction velocity levelled off at a value of 56 per cent of the value in Ringer's (18 m/sec, Fig. 38).

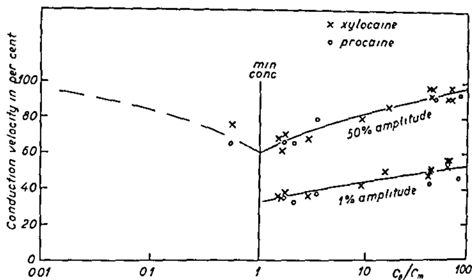


Fig 43 Correlation between conduction velocity and concentration of xylocaine and procaine base at 50 and 99 per cent block (22°C)
Ordinate conduction velocity in per cent of the value in Ringer s
Abscissa ratio between the base concentration of the anesthetic C_B and the minimum concentration C_m (Logarithmic scale)

The diminution in conduction velocity with xylocaine and procaine anesthesia.

The diminution in conduction velocity was determined in 13 experiments with xylocaine and in 6 with procaine anesthesia (Table 20)

At the same base concentration the conduction velocity decreased slightly more in the case of xylocaine than in the case of procaine (Figs 11 a, b) The tendency was clearest when small concentrations were used Expressing the base concentration in units of the minimum concentration for xylocaine and procaine the conduction velocity was reduced equally for xylocaine and procaine (Figure 13) That is, the minimum concentration of xylocaine and procaine reduced the conduction velocity to the same degree

The lower conduction velocity for xylocaine than for procaine at the same base concentration can be explained by the difference in minimum concentration and the effect of subminimal concentrations on conduction velocity For a given outer concentration a higher minimum concentration results in a greater number of active fibers exposed to high subminimal concentrations (Fig 12 a, c) Hence the conduction velocity is diminished most with the highest minimum concentration That for the same ratio between base concentration and minimum concentration the diminution in conduction velocity was the same for xylocaine and for procaine can be explained from the theoretical concentration distributions within the nerve (Fig 25) When

the outer concentration of xylocaine and procaine is the same multiple of the minimum concentration and when the number of blocked fibers is the same (i e the same reduction in action potential amplitude) also the same proportion of subminimally affected fibers is present

Discussion In the experiments described here it was found that the conduction velocity of the action potential was reduced in that portion of the nerve subjected to a local anesthetic Toman Woodbury and Woodbury (1947) demonstrated that block appeared when the conduction velocity had fallen to half its initial value Tasaki (1953) found that urethane diminished the conduction velocity in single fibers when the concentration was below the minimum concentration This alteration in conduction velocity occurred instantaneously after application of the local anesthetic

Since the conduction velocity returned to normal values in the post-anesthetic section of the nerve its decrease could not be due to a selective block of the most rapidly conducting fibers The explanation that the conduction velocity is diminished due to the effect of subminimal concentrations on all the fibers is supported by the finding of Tasaki (1953) that urethane in subminimal concentrations diminished the conduction velocity in single fibers With subminimal concentrations as the cause of the diminution in conduction velocity one can explain a) that the conduction velocity falls progressively with the progress of anesthesia b) that it falls most with concentrations of the anesthetic near the minimum concentration and c) that it decreases to the same degree with xylocaine and procaine in concentrations which are the same multiple of the minimum concentrations

CHAPTER 9

CLINICAL USE OF LOCAL ANESTHESIA AS RELATED TO THE FINDINGS ON ISOLATED NERVE

Although the isolated nerves investigated were not mammalian and the anesthetic effect was recorded on action potentials from mixed nerves some of the findings reported in this study may bear upon the clinical use of local anesthetics

In clinical use of local anesthesia when the anesthetic is applied to the nerve in situ there are a number of factors influencing the course of anesthesia which are more difficult to quantitate than in isolated nerve the concentration of the anesthetic depends on the rate of absorption the degree to which the anesthetic is bound to the tissue surrounding the nerve and in the case of procaine the degree of splitting caused by procaine esterase in the tissue The protolysis of the anesthetic is unknown since it is rare that the pH of the tissue after injection is known The anesthesia is carried out at a different temperature Finally the patients subjective reports of sensitivity to pain are the indicator for the effect of anesthesia and may be affected by psychic factors

Susceptibility of different types of nerve fibers to local anesthetics

In clinical practice local anesthetics are used in the main to block the *sensory fibers and particularly the pain fibers It is therefore of interest to* what extent local anesthetics affect these fiber types GOLDSCHEIDER (1886) found that sensory receptors vary in their threshold to cocaine in that the sensations of temperature pain pressure and touch disappear in that order

KOCHS (1886) DIXON (1905) and BORNINGHAUS and KOCHMANN (1929) demonstrated that local anesthetics applied to a mixed nerve block conduction along sensory fibers before the motor fibers are affected (DIXON s law) While motor fibers are predominantly rapidly conducting α fibers in the A group sensory impulses are conducted by A as well as B and C fibers at various velocities A pain sensation is experienced first as a rapid brief

intense pain conducted by A fibers followed by delayed pain conducted by the slow C fibers Since the C fibers form the majority GASSER (1943) considers them to be "the principal carriers of pain impulses" The experiments of HEINBECKER and BISHOP (1935) GASSER (1943) and LORENTE DE NÓ (1952) demonstrate that the slow C fibers are blocked before the B fibers which in turn are blocked before the A fibers According to GASSER (1943) the blocking time is directly proportional with fiber diameter, implying proportionality with conduction velocity That delayed pain is transmitted by the slowly conducting C fibers explains the fact that the sensation of pain disappears with local anesthesia before other sensory modalities are affected Since pain also is conducted by more rapid fibers quick pain would be expected to disappear later than delayed pain

The suitability of xylocaine and of procaine for clinical local anesthesia.

In the experiments reported here the course of anesthesia was only recorded as it affected the nerve fibers of the A group To obtain accurate information relevant to the disappearance of pain the minimum concentration of the anesthetic for C fibers should be known Assuming that the ratio between minimum concentration for A and for C fibers is the same for various local anesthetics it should be possible to determine from the present experiments whether xylocaine or procaine is best suited for clinical local anesthesia

Table 21

Time to half action potential amplitude ($t_{0.5}$) and to block ($t_{0.01}$) with 40 mM xylocaine and 40 mM procaine hydrochloride solutions at pH 7 5

	base concentration at pH 7 5 (C_B) mM	$t_{0.5}$ sec	$\frac{t_{0.5} \text{ procaine}}{t_{0.5} \text{ xylocaine}}$	$t_{0.01}$ sec	$\frac{t_{0.01} \text{ procaine}}{t_{0.01} \text{ xylocaine}}$
procaine	1 0	72	2 2	394	1 5
xylocaine	10 0	32		263	

mean values of $t_{0.5}$ and $t_{0.01}$ from
xylocaine experiments no 100 112 114 113 and 162 (Table 5)
procaine experiments no 142 143 147 and 148 (Table 6)

At the same base concentration (1 mM, p 81) the blocking time with xylocaine was 40 per cent longer than with procaine Because of the difference in ionization constants it is, however, necessary to use a 10 times higher concentration of procaine hydrochloride than of xylocaine hydrochloride to obtain the same base concentration at the same pH Therefore with the same amounts of procaine hydrochloride and of xylocaine hydrochloride at the same pH, xylocaine has a more rapid effect than procaine For example the time to half and to full block for 40 mM procaine and xylocaine hydrochloride solutions at pH 7 5 (corresponding to 1 mM procaine base and 10 mM xylocaine base) was 2 2–1 5 times less for xylocaine than for procaine anesthesia (Table 21)

Concentration and pH as related to the clinical effect of a local anesthetic.

With the base component as the active anesthetic factor, the time course of anesthesia can be affected by varying the hydrochloride concentration or the pH of the anesthetic solution From a clinical point of view it is of interest to determine how the variation in these two factors influences the course of anesthesia

The anesthetic effect of 20 and 40 mM xylocaine hydrochloride at pH 6–7 and 7 35 is shown in Table 22 Doubling the concentration of xylocaine hydrochloride at the same pH results in an 1 2–1 6 times shorter time to

Table 22

Time to half action potential amplitude ($t_{0.5}$) and to block ($t_{0.01}$) with xylocaine solutions of the same hydrochloride concentration but at different pH

hydrochloride concentration (C_0) mM	pH	base concentration (C_B) mM	$t_{0.5}$ sec	$t_{0.01}$ sec
20	6	0 21	315	1400
20	7	1 9	78	440
20	7 35	3 8	53	350
40	6	0 4	193	1000
40	7	3 7	54	350
40	7 35	7 5	37	290

$t_{0.5}$ and $t_{0.01}$ are calculated and interpolated from Fig 20

half and full block. A diminution in the pH of the anesthetic solution from 7.35 to 6 resulted in a 3.5–6 times slower fall in action potential amplitude.

To increase the effectiveness of the anesthetic solution it is therefore desirable to use a solution with high pH rather than to increase the concentration of the anesthetic. In clinical practice the solution usually has a pH of 3–5 with the aim of increasing the stability of adrenaline and procaine. It has been assumed that the buffering power of the tissue was sufficient to change the pH of such a solution to about 7.4 almost instantaneously. In support of this assumption TAINTER and MOOSE (1938) found that an acid procaine solution was neutralized by the addition of small amounts of serum. On the other hand BJORN (1947) and HOLLER (1953) measured the change in pH in living tissue infiltrated with various local anesthetics and found that the tissue pH remained subnormal for some time. For example after injection of 1 ml of 2 per cent xylocaine and procaine hydrochloride solution at pH 3 the pH of the tissue did not reach normal until after 45 minutes (BJORN 1947). Similarly HOLLER (1953) found that after injection of 0.75 ml of 2 per cent procaine hydrochloride at pH 6.23 the pH of the tissue did not return to 7.3 until after 45 minutes.

In a clinical study TAINTER et al. (1938, 1939, 1941) could not demonstrate any difference in the effect of procaine solutions at various pH. Similarly the pH of a xylocaine solution was not of importance for the occurrence of anesthesia, its extent and its duration (HULDT 1953). No information is given, however, as to the pH dependence of the latency of anesthesia and the investigations were carried out solely with high concentrations of the local anesthetics. On the other hand, HARNISCH (1956) found that procaine solutions were clinically more effective at pH about 7.4 than at pH 5.

A number of clinical experiences with local anesthesia may be explained from the effect of pH on the protolysis of the anesthetic. In topical anesthesia of the mucous membranes the effect is often unsatisfactory. This may be due to the lack of buffer at the tissue surface which can neutralize the anesthetic solution. It has therefore been recommended that a base be added to the anesthetic solution before it is used for surface anesthesia. Local anesthetics have also less effect in infected tissues probably due to their more acid reaction (LETTERER 1959) preventing the anesthetic from being neutralized. The action of a local anesthetic is often unsatisfactory in traumatized tissue (JORGENSEN 1956). In this case the same change occurs as during infection, i. e. the pH of the tissue is diminished. Therefore local anesthetics are most effective when injected slowly, since rapid injection may traumatize the tissue and cause a diminution in pH.

The surprisingly unsatisfactory anesthesia often associated with the in-

9*

jection of large doses of local anesthetic (JORGENSEN 1956) may be due in part to traumatization of the tissue and in part to the insufficient buffer capacity of the tissue to neutralize the anesthetic. The same line of reasoning can explain the cases when, after an insufficient nerve block, one places a new depot of the anesthetic around the nerve and still obtains no better anesthesia. LOVENSTEDT (1911) recommends in such cases that higher concentrations be used with the second than with the first injection. In view of the fact that the *failure is due to the effect of pH it might be more suitable to* inject a basic solution instead of using higher concentration.

The addition of adrenaline or other vasoconstrictors is usual in the clinical use of local anesthetics. According to BJORN (1947) and HOLLER (1953) this involves a further prolongation of the time required for neutralization of xylocaine and procaine in the tissue possibly because the ischemia diminishes the buffer capacity of the tissue. In addition HILLE and TESKI (1956) believe that adrenaline releases lactic acid. Both explanations imply that the base concentration of the anesthetic is increased only slowly and thereby the latency for the onset of anesthesia is prolonged.

If the anesthetic can remain in contact with the nerve for a sufficiently long time, a small concentration near the minimum concentration is sufficient *to obtain full anesthesia even if the time required may be long* in that the solution must first be neutralized and the diffusion time into the nerve is *long when small concentrations are used. It is of advantage if the pH of* the anesthetic solution is high when it is injected: the anesthesia begins more rapidly, an effect is obtained with a smaller concentration and an *active concentration is effective for a longer time.* That smaller concentrations are sufficient at high pH is of special importance in view of the progressively increasing toxicity with increasing concentration (GOLDBERG 1947).

The effect of the concentration of the anesthetic and of hydrogen ions on the duration of anesthesia.

From the restitution time of the nerve after local anesthesia (Chapter 7) certain conclusions may be drawn as to how the anesthetic influences the duration of anesthesia. To obtain an anesthesia of short duration an anesthetic should be chosen with a high minimum concentration, a high velocity factor and a small thermodynamic ionization constant. The anesthetic solution should be in low concentration and at high pH.

Since the unbound base is the active anesthetic component and the acid component is ineffective one might shorten the duration of anesthesia by transforming the base component to acid in the nerve by lowering the pH

of the nerve This has however the disadvantage that a lower pH not only reduces the base concentration but also the minimum concentration and thereby the base component at low pH becomes more active anesthetically The following example demonstrates that this procedure is not practicable

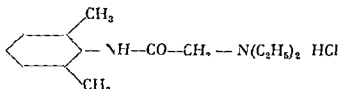
The lowest pH value at which hemolysis does not occur is 5 7 (CHRISTOPHER 1929) A 1 per cent (40 mM) xylocaine hydrochloride solution which contains 6 6 mM xylocaine base at pH 7 3 contains 0 21 mM base at 5 7 pH This base concentration is however greater than the minimum concentration at pH 5 7, and for this reason the solution still has an anesthetic effect

APPENDIX 1

CHEMICAL AND PHYSICAL DATA FOR XYLOCAINE AND PROCAINE

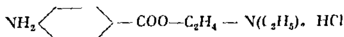
Xylocaine (lidocaine lignocaine, leostesin) Synthesized by LOFGREN (1943) who (1948) gives the following data

structural formula



molecular weight of the base 234 3
molecular weight of the hydrochloride 270 8
thermodynamic ionization constant (K_a^{25}) $1\ 40 \times 10^{-8} = 10^{-7\ 85}$
solubility Astra gives the information that the solubility of xylocaine base in water at 25°C is 0 4 per cent = 17 mM Since there was used at most 15 5 mM xylocaine base in the experiments reported here the xylocaine base was completely dissolved

Procaine (novocaine)
Synthesized by EINHORN (1905)
structural formula



molecular weight of the base 236 3
molecular weight of the hydrochloride 272 77
thermodynamic ionization constant (K_a^{25}) $10^{-8\ 93}$ (calculated from EISENBRAND and PICHER (1938) see p 28)
solubility EISENBRAND and PICHER (1938) found the solubility of procaine base to be 0 13 per cent at 25°C = 5 5 mM Since the maximal procaine base concentration used in these experiments was 2 01 mM the whole amount was dissolved

PREPARATION OF RINGER'S AND ANESTHETIC SOLUTIONS

All Ringer's and anesthetic solutions used in the experiments were isotonic (osmotic pressure corresponding to 219 m-equiv) and contained calcium, potassium and glucose in concentrations corresponding to their concentration in normal frog Ringer's as well as 75 mM sodium. The solutions were prepared from 6 stock solutions to obtain concentrations between 0 and 10 mM xylocaine or procaine hydrochloride at pH 5.5 to 7.8.

To prepare the anesthetic solutions 2 per cent xylocaine or procaine hydrochloride solutions were used (stock solution 6).

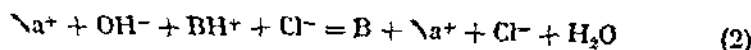
Xylocaine hydrochloride solution*) is a watery solution to which no disinfectant was added as to the commercial solutions for clinical use. An analysis carried out by "Astra" of the solution used showed that the concentration of xylocaine hydrochloride in the solution was 1.946 per cent.

Procaine hydrochloride solution. Unlike xylocaine which is stable in solution, procaine is hydrolyzed in a watery solution to para amino-benzoic acid and diethyl amino-ethanol. The disintegration occurs more rapidly at high pH; the maximal stability is at pH 3.6. Since the splitting is a monomolecular process the time course of the hydrolysis may be described as

$$x = a(1 - e^{-ct}) \tag{1}$$

where c is the velocity constant which varies with pH, t is the time in hours, a is the concentration of procaine at the onset and x is that portion which is hydrolyzed after time t. From the velocity constant at various pH levels (TLRP 1919) it is seen that procaine is hydrolysed at a rate of 2.3 per cent a day (20°C, pH 7.35). The procaine solution was therefore prepared from crystalline procaine immediately before each experiment.

The osmotic pressure of xylocaine or procaine hydrochloride (denoted BHCl) depends on the degree of protolysis of BH^+ and therefore on pH. In acid solutions BH^+ predominates which, with Cl^-, contributes 2 osmotically active ions. If the solution is rendered alkaline, for example by the addition of NaOH



$$Na^+ + OH^- + BH^+ + Cl^- = B + Na^+ + Cl^- + H_2O \tag{2}$$

3 osmotically active particles are formed: B (the uncharged base of the anesthetic), Na^+ and Cl^-. It can therefore be calculated how many m-equiv a

*) The xylocaine solution was kindly made available by "Astra".

certain concentration of anesthetic substance constitutes by multiplying the concentration of hydrochloride by 2 and adding the number of hydroxyl ions which, at the given pH, react with BH^+. The amount of hydroxyl ions is the same as that of the free base formed (see p. 27 and Fig. 3).

To exemplify the preparation of the special Ringer's and of the anesthetic solutions, the constituents of 100 ml of 40 mM xylocaine Ringer's solution are described.

***Stock solution b** contained 2 per cent xylocaine hydrochloride.* According to Table 23, 54 ml of this stock solution should be used.

Table 23

Amount of xylocaine and procaine hydrochloride in the anesthetic solutions

xylocaine hydrochloride concentration		amount of 2% xylocaine HCl per 100 ml solution	procaine hydrochloride concentration		amount of 2% procaine HCl per 100 ml solution
mM	%	ml	mM	%	ml
1	0.027	1.35	1	0.027	1.37
2	0.054	2.7	2	0.055	2.73
5	0.135	6.75	5	0.136	6.83
20	0.54	27.0	20	0.546	27.3
40	1.08	54.0	40	1.09	54.6

Table 24

Milliequivalents per liter contributed by a 6.6 mM phosphate buffer at different pH

pH	mM		m equiv		
	concentration of Na_2HPO_4*)	concentration of NaH_2PO_4	Na_2HPO_4	NaH_2PO_4	total
5.5	0.33	6.27	0.99	12.54	13.53
6	0.87	5.73	2.61	11.46	14.07
6.5	2.15	4.45	6.45	8.90	15.35
7	3.95	2.65	11.85	5.30	17.15
7.5	5.52	1.08	16.56	2.16	18.72
7.8	6.00	0.60	18.00	1.20	19.20

*) concentration of Na_2HPO_4 is equal to the concentration of NaOH in Fig. 5

Stock solution 1. 25 ml of this solution contribute to the anesthetic solution 2.7 mM KCl (5.4 m-equiv), 1.8 mM $CaCl_2 \cdot 6H_2O$ (5.4 m-equiv), 1.1 mM glucose (1.1 m-equiv) and 6.6 mM $NaH_2PO_4 \cdot 2H_2O$. Because the phosphate milliequivalents are included in the calculation as phosphate buffer (see Table 24) stock solution 1 contributes 11.9 m-equiv.

In some of the experiments 30 g/l of Dextran were added to the stock solution 1 to add colloid osmotic pressure to the solutions. Since it was found that the addition of Dextran had no influence on the results, this was omitted in the subsequent experiments.

Stock solution 2 contained 0.2 M sodium hydroxide. Sodium hydroxide is added in an amount which, with NaH_2PO_4 from stock solution 1, gives a buffer of the desired pH. Table 24 indicates the concentration of sodium hydroxide (= concentration of Na_2HPO_4) to be added to the solution for this purpose as well as the osmotic pressure the phosphate buffer contributes at various pH levels. In the case of the anesthetic solution there must in addition be added an amount of sodium hydroxide which reacts with the anesthetic acid at the given pH, in other words the same amount which is formed as free anesthetic base (see p. 136). Table 25 shows how much sodium hydroxide should be added to a 40 mM xylocaine Ringer's solution at different pH levels.

Table 25

NaOH to be added to a 40 mM xylocaine Ringer's with 6.6 mM phosphate buffer at different pH

pH	NaOH reacting with phosphate buffer*)	NaOH reacting with 40 mM xylocaine HCl**)		total NaOH	ml 0.2 M NaOH per 100 ml solution
	mM	mM	m-equiv	mM	
5.5	0.33	0.13	0.13	0.46	0.23
6	0.87	0.41	0.41	1.29	0.64
6.5	2.15	1.26	1.26	3.41	1.72
7	3.95	3.72	3.72	7.67	3.84
7.5	5.52	9.78	9.78	15.3	7.6
7.8	6.00	15.49	15.49	21.5	10.7

*) values from Fig 5

**) according to equation (2) p 135. The concentration of NaOH = the concentration of free base given in Fig 3 at various pH

Stock solution 3 contained 5 per cent $NaHCO_3$ Table 26 shows how much must be added to an anesthetic solution saturated with 3 per cent carbon dioxide to achieve pH 5 5 to 7 8 in the sodium bicarbonate carbon dioxide buffer Furthermore it may be seen how many milliequivalents per liter the sodium carbonate solution contributed

Table 26

Amount of $NaHCO_3$ to be added to an anesthetic or a Ringer s solution (ionic strength 0 134) saturated with 3 per cent carbon dioxide at various pH

pH	$NaHCO_3$ mM	$NaHCO_3$ m equiv	n l 5 % $NaHCO_3$ per 100 ml solution
5 5	0 2	0 4	0 04
6	0 7	1 4	0 11
6 5	2 0	4 0	0 35
7	6 6	13 2	1 11
7 5	21 0	42 0	3 53
7 8	42 0	84 0	7 06

Stock solution 4 contained 0 1 M sodium chloride This was added to supplement the already added sodium from solutions 1 2 and 3 to raise the sodium concentration of the anesthetic solution to 75 mM From Table 27 it may be seen how many mM sodium must be added to the anesthetic solution at various pH levels Table 28 demonstrates how many milliequivalents per liter the sodium chloride solution contributed and how many ml of stock solution 4 must be added to prepare 100 ml xylocaine Ringer s at different pH levels

Table 27

Sodium (mM) added to a 40 mM xylocaine Ringer s from the different stock solutions and sodium chloride (mM) to be added to obtain a total concentration of 75 mM sodium

	pH	5 5	6	6 5	7	7 5	7 8
Table 25 stock solution 1 Table 26	NaOH	0 16	1 28	3 41	7 67	15 2	21 5
	NaH_2PO_4	6 6	6 6	6 6	6 6	6 6	6 6
	$NaHCO_3$	0 2	0 7	2 0	6 6	21 0	42 0
	NaCl	67 74	66 42	62 99	54 13	32 2	4 9
	total	75 00	75 00	75 00	75 00	75 00	75 00

Table 28

Amount of sodium chloride to be added to a 40 mM xylocaine Ringer's at various pH

pH	mM (from Table 27)	m equiv	ml 0.4 M sodium chloride per 100 ml solution
5.5	67.74	135.5	16.8
6	66.42	133.0	16.6
6.5	62.99	126.0	15.7
7	54.13	108.2	13.5
7.5	32.2	64.4	8.0
7.8	4.9	9.8	1.2

Stock solution 5 contained 0.5 M choline chloride which was added to increase the osmotic pressure to 249 m-equiv Table 29 indicates how many m-equiv of choline chloride must be added to the xylocaine Ringer's solution at different pH levels Table 30 shows how many mM choline chloride as well as how much of stock solution 5 must be used to prepare 100 ml xylocaine Ringer's at different pH levels

Table 29

Milliequivalents per liter added to 40 mM xylocaine Ringer's from the different stock solutions and milliequivalents choline chloride per liter to be added at various pH to obtain a total osmotic pressure corresponding to 249 m equiv

from Table	pH	5.5	6	6.5	7	7.5	7.8
	xylocaine HCl 40 mM	80.0	80.0	80.0	80.0	80.0	80.0
25	Na^+ reacting with xylocaine HCl	0.13	0.41	1.20	3.72	9.78	15.49
	stock solution 1	11.9	11.9	11.9	11.9	11.9	11.9
24	phosphate buffer	13.5	14.1	15.4	17.2	18.7	19.2
26	bicarbonate buffer	0.4	1.4	4.0	13.2	42.0	84.0
28	NaCl	135.5	133.0	126.0	108.2	64.4	9.8
	choline chloride	7.57	8.19	10.44	14.78	22.22	28.61
	total	249.0	249.0	249.0	249.0	249.0	249.0

Table 30

Choline chloride to be added to 40 mM xylocaine Ringer's at various pH

pH	m equiv (from Table 29)	mM	ml 0 5 M choline chloride per 100 ml solution
5 5	7 57	3 8	0 8
6	8 19	4 1	0 8
6 5	10 44	5 2	1 0
7	14 78	7 4	1 5
7 5	22 22	11 1	2 2
7 8	28 61	14 3	2 9

To prepare 100 ml of anesthetic solution at concentrations of 5, 20 or 40 *mM xylocaine hydrochloride at every pH level between 5 5 and 7 8 curves* have been drawn (Figs 44 A, B, C) from the values found in Tables 25, 28, 30 Though the tables were intended for the preparation of xylocaine solution, the values could also be used to prepare procaine solutions with a 1 per cent error As an example Table 31 indicates the components of 100 ml 40 mM of xylocaine Ringer's solution at pH 7

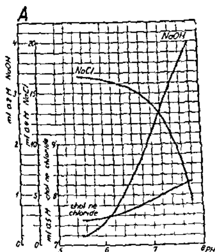
Table 31

An example of the composition of 100 ml of anesthetic solution at pH 7 made up from 6 stock solutions (*40 mM xylocaine Ringer's*)

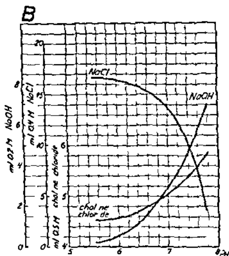
stock solution	content	from	added amount in ml
1	various salts		25 0
2	0 2 M NaOH	Fig 44 C	3 8
3	5 % $NaHCO_3$	Fig 44 D	1 1
4	0 4 M NaCl	Fig 44 C	13 2
5	0 5 M choline chloride	Fig 44 C	1 3
6	2 % xylocaine hydrochloride	Table 23	54 0
		total	98 4 ml

+ aqua destillata ad 100 ml

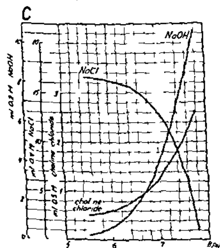
The preparation of the Ringer's solution followed the same principles 25 ml of stock solution 1 were used for every 100 ml of Ringer's solution Figure 44 D shows how much must be used of the stock solutions 2 3, 4 and 5 to obtain solutions at various pH



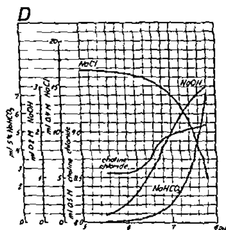
5 mM xylocaine Ringer s



20 mM xylocaine Ringer's



40 mM xylocaine Ringer s



Special Ringer s

Fig 41 The amount of stock solutions 2 4 and 5 to obtain 100 ml solution with (*A*) 5 (*B*) 20 and (C) 40 mM xylocaine hydrochloride and (*D*) of special Ringer's a given *pH* a sodium concentration of 75 mM and an osmotic pressure correspond ng to 249 m-equiv The amount of stock solution 3 (sodium bicarbonate) for all solutions is shown in (*D*) Ordinate ml stock solution
Abscissa pH

Table 30.

Choline chloride to be added to 40 mM xylocaine Ringer's at various pH

pH	m equiv (from Table 29)	mM	ml 0.5 M choline chloride per 100 ml solution
5.5	7.57	3.8	0.8
6	8.19	4.1	0.8
6.5	10.44	5.2	1.0
7	14.78	7.4	1.5
7.5	22.22	11.1	2.2
7.8	28.61	14.3	2.9

To prepare 100 ml of anesthetic solution at concentrations of 5, 20 or 40 mM xylocaine hydrochloride at every pH level between 5.5 and 7.8 curves have been drawn (Figs 41 A, B, C) from the values found in Tables 25, 28–30. Though the tables were intended for the preparation of xylocaine solution the values could also be used to prepare procaine solutions with a 1 per cent error. As an example Table 31 indicates the components of 100 ml 40 mM of xylocaine Ringer's solution at pH 7.

Table 31

An example of the composition of 100 ml of anesthetic solution at pH 7 made up from 6 stock solutions (40 mM xylocaine Ringer's)

stock solution	content	from	added amount in ml
1	various salts		25.0
2	0.2 M NaOH	Fig 41 C	3.8
3	5% $NaHCO_3$	Fig 41 D	4.1
4	0.4 M NaCl	Fig 41 C	13.2
5	0.5 M choline chloride	Fig 41 C	1.3
6	2% xylocaine hydrochloride	Table 23	51.0
		total	98.4 ml

+ aqua destillata ad 100 ml

The preparation of the Ringer's solution followed the same principles. 25 ml of stock solution 1 were used for every 100 ml of Ringer's solution. Figure 41 D shows how much must be used of the stock solutions 2, 3, 4 and 5 to obtain solutions at various pH.

where J_0 and J_1 are Bessel functions of the first kind while I_0 is a modified Bessel function β_n are the zeros for J_0.

To evaluate the effect of a breakdown of the substance on the course of diffusion, let us compare expression (4) for the distribution of concentration in the cylinder at various times with the expression (cf Chapter 5) which disregarded hydrolysis

$$C(r,t) = C_e\left\{1 - 2\sum_{n=1}^{\infty} e^{-\frac{D\beta_n^2 t}{r_0^2}} \frac{J_0\left(\beta_n \frac{r}{r_0}\right)}{\beta_n J_1(\beta_n)}\right\} \tag{5}$$

It is seen from (4) and (5) that the hydrolysis does not influence the course of diffusion significantly as long as the hydrolysis constant s fulfils the following conditions

$$\frac{I_0(r\sqrt{s/D})}{I_0(r_0\sqrt{s/D})} \approx 1 \tag{6}$$

and

$$s \ll \frac{D\beta_n^2}{r_0^2}, \ n = 1, 2, 3 \tag{7}$$

It appears from (4) that condition (6) concerns the final outcome of the diffusion, i e the concentrations obtained at time infinity, while conditions (7) are concerned with the time course of diffusion If these conditions are not fulfilled the course of diffusion with simultaneous chemical reaction diverges from the course of pure diffusion and the concentration within the cylinder never becomes the same as outside it

The spontaneous hydrolysis of procaine amounts to 0 25 per cent per hour at pH 7 8 (TI RP 1949 and SKOU 1951) This corresponds to the following value for the hydrolysis constant s in (1)

$$s = 7 \times 10^{-7} \text{ per cm}^2 \text{ and sec} \tag{8}$$

Let us insert this value in conditions (6) and (7) Since $I_0(r\sqrt{s/D})$ increases as r increases, condition (6) will be fulfilled if

$$\frac{I_0(0)}{I_0(r_0\sqrt{s/D})} \approx 1$$

in other words if

$$I_0(r_0\sqrt{s/D}) \approx 1 \tag{9}$$

since $I_0(0) = 1$

With $D = 2\,1 \times 10^{-7}$ cm²/sec, with $r_0 = 0\,1$ mm and with $s = 7 \times 10^{-7}$ cm^{-2} × sec^{-1} it follows that

APPENDIX 2

DIFFUSION WITH SIMULTANEOUS INACTIVATION

by P. Rosenfalck

In the mathematical description of the course of anesthesia in whole nerve (Chapter 5) it was not taken into account that there might be a loss of the anesthetic in the nerve on account of chemical reaction. Since procaine undergoes spontaneous non-enzymatic hydrolysis (Terp 1919, Skou 1951) and possibly hydrolysis in the nerve caused by procaine esterase (Nordqvist 1952 a) it was necessary to evaluate the influence of a hydrolysis on the theoretical course of anesthesia.

Let us therefore consider an infinitely long cylinder with radius r_0 placed in a large volume containing a substance in concentration C_e which diffuses into the cylinder with an effective diffusion coefficient D. Let us furthermore assume that the substance to a certain extent is metabolised in the cylinder and that the amount metabolised per cm^3 and per second is proportional to the concentration of the substance. The course of diffusion is then determined by the following equation

$$\frac{\partial C}{\partial t} + s \times C = D \left[\frac{\partial^2 C}{\partial r^2} + \frac{1}{r} \frac{\partial C}{\partial r} \right], \quad \begin{array}{c} 0 < r < r_0 \\ t > 0 \end{array} \qquad (1)$$

with the boundary conditions

$$C = C_e \quad \text{for } r = r_0 \qquad (2)$$

$$C = 0 \quad \text{for } t = 0 \quad \text{and} \quad 0 \leq r \leq r_0 \qquad (3)$$

In (1) $C = C(r,t)$ represents the concentration of the diffusing substance at distance r from the axis of the cylinder to time t, $s \times C$ represents the amount of the substance metabolized per cm^3 and per second.

The differential equation (1) may be solved by the use of the Laplace-transform. The solution which fulfils the boundary conditions (2) and (3) is

$$C(r,t) = C_e \left[\frac{I_0 (r\sqrt{s/D})}{I_0 (r_0\sqrt{s/D})} - 2 \sum_{n=1}^{\infty} e^{-\left(\frac{D\beta_n^2}{r_0^2} + s\right)t} \frac{J_0\left(\beta_n \frac{r}{r_0}\right)}{\left(\beta_n + \frac{s r_0^2}{D\beta_n}\right) J_1(\beta_n)} \right] \qquad (4)$$

APPENDIX 3

RESTITUTION OF THE NERVE AFTER ANESTHESIA IN TERMS OF DIFFUSION THEORY

by P ROSENFALCK

In Chapter 6 it was shown that the progress of anesthesia in whole nerve as evidenced by the gradual diminution of its action potential can be described with good approximation assuming that the anesthetic diffuses into the nerve with a velocity factor D and that there is a minimum blocking concentration C_m It was therefore of interest to investigate wheter the gradual restitution of the nerve from anesthesia can be described on the same basis

Considering the nerve as an infinitely long cylinder with radius r_0 the distribution of the anesthetic in the nerve at various times during the progress of anesthesia was given by (Chapter 5)

$$C_A(r\ t) = C_e\left[1 - 2\sum_{n=1}^{\infty} e^{-\frac{D\beta_n^2 t}{r_0^2}} \frac{J_0\left(\beta_n \frac{r}{r_0}\right)}{\beta_n J_1(\beta_n)}\right] \tag{1}$$

with C_e representing the outer concentration of the anesthetic and t the time the nerve has been exposed to this concentration

Let us assume that the anesthesia is continued until the moment when the nerve is fully anesthetized i e when the concentration of the anesthetic at the nerve axis has just reached the minimum concentration C_m Denoting by $C_A(r\ t)$ the concentration of the anesthetic in the nerve during the development of anesthesia this implies that the anesthesia is continued to the time t_0 determined by the following equation

$$C_A(0\ t_0) = C_m \tag{2}$$

If the nerve is thereafter placed in Ringer s solution and if the anesthetic is assumed to diffuse gradually out of the nerve at a rate determined by the velocity factor of the progress of anesthesia then the course of restitution is described by the following differential equation

1

$$r_0\sqrt{s/D} = 0.07$$

Since I_0 (0 07) = 1.0012 conditions (9) and (6) are fulfilled This indicates that the splitting of procaine causes no more than a 0 1 per cent lower concentration in the centre of the nerve than outside the nerve when stationary conditions have been reached

Conditions (7) which concern the time course of anesthesia are with the values of s, D and r_0 given above.

$$7 \times 10^{-7} \ll \frac{1.5 \times 10^{-7} \times \beta_n^2}{16 \times 10^{-4}}$$

or

$$\beta_n^2 \gg 7.5 \times 10^{-3} \qquad (10)$$

Since β_n increases with increasing n, and since $\beta_1 = 2.4$ these conditions are also fulfilled.

Therefore, the spontaneous hydrolysis of procaine is so slow that it does *not influence the time course of the diffusion of procaine into the nerve*. Even a 10 times greater splitting due to an effect of procaine esterase would not influence the time course of procaine anesthesia.

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Let us assume that the anesthesia is continued until the moment when the nerve is fully anesthetized, i e when the concentration of the anesthetic at the nerve axis has just reached the minimum concentration C_m. Denoting by $C_A(r\ t)$ the concentration of the anesthetic in the nerve during the development of anesthesia this implies that the anesthesia is continued to the time t_0 determined by the following equation

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$$\frac{\partial C}{\partial t} = D\left[\frac{\partial^2 C}{\partial r^2} + \frac{1}{r}\frac{\partial C}{\partial r}\right], \quad \begin{matrix} 0 < r < r_0 \\ t > t_0 \end{matrix}$$

with the boundary conditions

$$C(r_0, t) = 0 \text{ for } t > t_0$$

and

$$C(r, t_0) = C_A(r, t_0)$$

The solution which fulfils these boundary conditions is

$$C(r,t) = 2C_e \sum_{n=1}^{\infty} \frac{J_0\left(\beta_n \frac{r}{r_0}\right)}{\beta_n J_1(\beta_n)} \left\{ e^{-\frac{D\beta_n^2 (t-t_0)}{r_0^2}} - e^{-\frac{D\beta_n^2 t}{r_0^2}} \right\} \quad ($$

where t_0 according to (1) and (2) is determined by

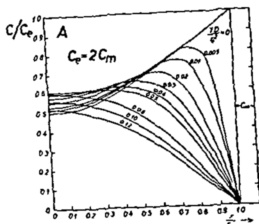
$$C_n = C_e\left[1 - 2\sum_{n=1}^{\infty} \frac{e^{-\frac{D\beta_n^2 t_0}{r_0^2}}}{\beta_n J_1(\beta_n)}\right] \quad ($$

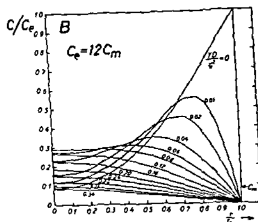
From (1) and (6) it is seen that the distribution of concentrations durin restitution may be expressed as a difference between two concentratio distributions during the development of anesthesia

$$C(r, t) = C_A(r, t) - C_A(r, t - t_0), \quad t > t_0 \quad (8$$

Utilizing the $C_A(r, t)$ curves given by CARSLAW and JAEGER (1947) (Fig. 2) and equation (8) the time course of restitution was calculated for variou concentrations of anesthetic. In Figure 15 A, B and C are shown exampl of the distribution of concentrations at various times after the nerve wa transferred from the anesthetic solution to Ringer's solution. These distri butions illustrate that early in restitution there is diffusion toward th nerve axis simultaneous with diffusion out of the nerve (p. 109).

From the distribution of concentrations the fraction of the cross-sectiona area of the nerve was calculated in which the concentration of the anesthetic was less than the minimum concentration (cf. p. 86). This fraction was assumed to be a measure of the action potential amplitude. The theoretical restitution courses obtained in this way for various concentrations are compared with the experimental findings in Figure 16. In agreement with the experimental findings restitution progresses faster with small than with large concentrations of anesthetic. The experimentally measured restitution





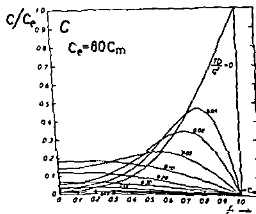


Fig 45 Calculated distributions within the nerve of concentration of the anesthetic during restitution from anesthesia with different base concentrations

Ordinate concentration (C) in units of the outer concentration of the anesthetic (C_e)

Abscissa distance (r) from the nerve axis in units of the nerve radius (r_0)

The figures on the curves denote the restitution time measured from the time when the anesthetic was substituted by Ringer's given in terms of $TD\,r_0^2$ where T is the time after removal of the anesthetic and D the diffusion coefficient C_m is the minimum concentration

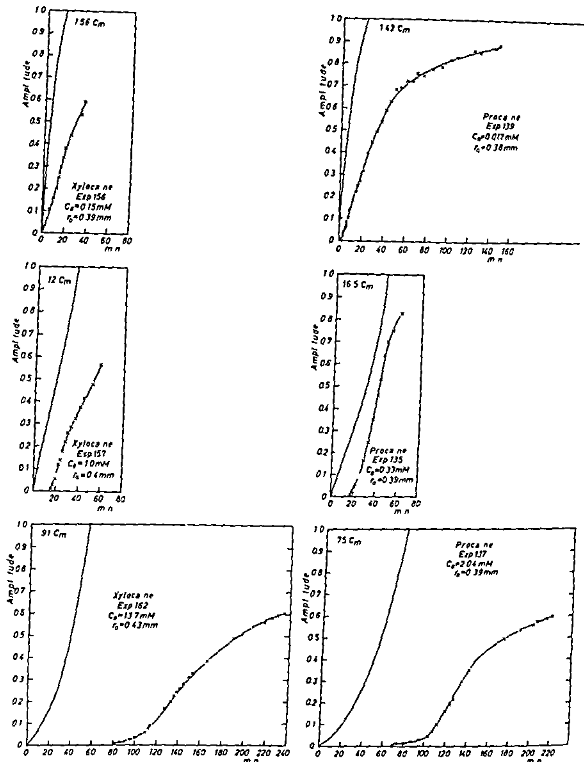


Fig. 46 Experimental and calculated time course of restitution after anesthesia with various concentrations of xylocaine and procaine (given in units of the minimum concentration) The left curve in each diagram is calculated

Ordinate action potential amplitude in units of amplitude in Ringer's before anesthesia of the nerve

Abscissa minutes from the moment when the anesthetic solution was replaced by Ringer's

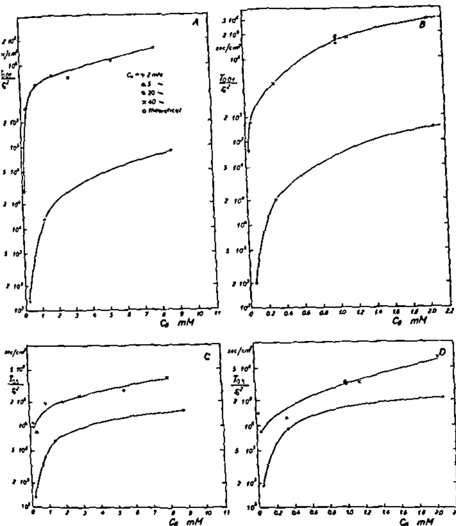


Fig 47 Experimental and calculated times for 1 ($T_{0.01}$) and 50 ($T_{0.5}$) per cent restitution after anesthesia as a function of the base concentration of the anesthetic The upper curve in each diagram is experimental.

Ordinate $T_{0.01}$ and $T_{0.5}$ in terms of T/r_0^2 to compensate for differences in nerve radius (r_0)

Abscissa base concentration of the anesthetic in mM

A $T_{0.01}$ xylocaine, B $T_{0.01}$ – procaine

C $T_{0.5}$ – xylocaine, D $T_{0.5}$ – procaine

The symbols indicate hydrochloride concentrations (C_0)

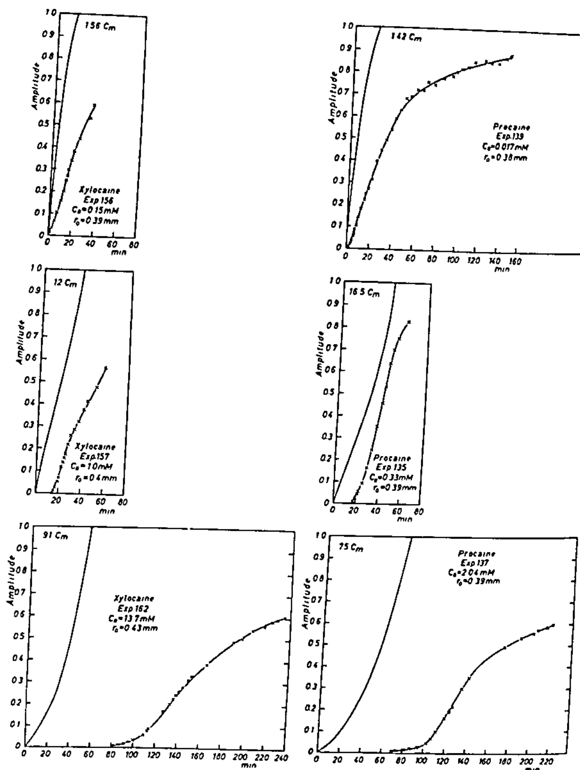


Fig 46 Experimental and calculated time course of restitution after anesthesia with various concentrations of xylocaine and procaine (given in units of the minimum concentration) The left curve in each diagram is calculated.

Ordinate. action potential amplitude in units of amplitude in Ringer s before anesthesia of the nerve.

Abscissa: minutes from the moment when the anesthetic solution was replaced by Ringer's.

SUMMARY

The time course of local anesthesia (xylocaine and procaine) and the concomitant change in conduction velocity were studied in isolated frog nerve.

The time course of local anesthesia depends on the ratio between the concentration of the active component of the anesthetic and the smallest concentration just sufficient to block the nerve (minimum concentration). The minimum concentration was determined experimentally and it was investigated whether the uncharged base component of the anesthetic was the active portion of the molecule.

The anesthetic penetrates into the nerve by diffusion and it has been investigated to what extent the laws of diffusion can account for the time course of local anesthesia and if it is justified to calculate a minimum concentration and a diffusion coefficient assuming free diffusion of the anesthetic into the nerve (EHRENBERG 1948).

From the time course of the restitution of the nerve impulse after local anesthesia an attempt was made to clarify the factors which determine the course of restitution.

The role of the nerve sheath for the time course of anesthesia was studied by comparing the time course in nerves with and without sheath. To study the effect of subminimal concentrations of the local anesthetics on the nerve fiber membrane the conduction velocity of the nerve impulse was determined during the course of anesthesia.

The relationship of the experimental results to clinical local anesthesia is discussed.

Method.

In most instances the action of a local anesthetic has been investigated by psycho-physical methods or by using the mechanical response of a nerve-muscle preparation as indicator (EHRENBERG 1948, SKOU 1954). These procedures do not allow the time course of anesthesia to be followed with sufficient accuracy. Nor do they permit an evaluation of the influence of the

lasted, however, considerably longer than to be expected from theory in that the theory does not account for the increasing delay in the onset of restitution with increasing concentration. The times to 1 per cent and 50 per cent restitution were compared with the experimentally found values at various concentrations of xylocaine and procaine (Figure 47 A, B, C and D). The theoretical restitution times were calculated by introducing the experimentally determined minimum concentration and the 'velocity factor' (cf. Chapter 6) in equation (8) and the restitution curves derived from it. The experimental times to 1 per cent restitution of the action potential were 20–40 times longer than the theoretical (Figure 47 A and B) and the experimental times to 50 per cent restitution were 2–3 times longer than the theoretical (Figure 47 C and D). In spite of these disagreements between theory and experiment the calculations have given evidence that restitution after anesthesia of a whole nerve is determined to a considerable degree by *diffusion of the anesthetic out of the nerve.* *The cause of the discrepancy* between experiment and theory is probably the binding of the local anesthetic to the nerve tissue. Such binding would exert its largest effect at the onset of restitution.

On the other hand, the calculations presented in this section have shown that even with simple diffusion restitution takes much longer than block, the difference being 4 and 12 times respectively with concentrations which are 12 and 80 times the minimum concentration. It is therefore not justified to consider the fact that complete restitution after anesthesia often takes considerably longer than block as an indicator of binding of the anesthetic to nerve tissue (BENNETT et al. 1942).

same concentration of calcium, potassium and sodium This was achieved by reducing the concentration in the Ringer s solution of sodium chloride to 75 mM and by adding the anesthetic or choline chloride until the osmotic pressure corresponded to 249 m equiv

The effect of a local anesthetic on action potential amplitude.

When the nerve was subjected to a local anesthetic the action potential amplitude fell instantaneously As anesthesia progressed the amplitude diminished more slowly The anesthetic effect of xylocaine and procaine hydrochloride was accelerated with increasing concentration and at increasing pH Both an increase in hydrochloride concentration and in pH cause an increase in the base concentration of the anesthetic A fourfold increase in base concentration with unaltered acid concentration reduced the time course of anesthesia by more than 40 per cent An increase in acid concentration by up to 4 7 times did not affect the time course of anesthesia as long as the base concentration was constant Thus it seems likely that the base component of the anesthetic molecule is the anesthetically active factor

A comparison of the course of anesthesia with xylocaine and procaine showed that anesthesia progressed twice as rapidly with xylocaine as with procaine at the same hydrochloride concentration and pH This was due to the fact that the dissociation constant of xylocaine hydrochloride was 10 times higher than that of procaine hydrochloride

In *clinical* use when the most rapid effect and the least toxicity are required one should use a solution with as high a pH as possible rather than a high hydrochloride concentration

The minimum concentration was determined from the smallest concentration capable of blocking a nerve after at least 6–8 hours exposure This time of exposure was chosen since the excitability decreases when the isolated nerve remains for 12 24 hours in Ringer s solution (Bornsnoh l s and Kochmann 1927) Experiments with concentrations only slightly above the minimum concentration of the local anesthetic indicated that an exposure time of 6–8 hours allowed equilibrium In agreement with Skou (1951) it was found that the minimum concentration for procaine was greater when the pH of the anesthetic solution was increased The same relationship held in the case of xylocaine base The minimum concentration for xylocaine base was 0 01 mM at pH 6 00 and 0 11 mM at pH 7 10 In the case of procaine base the following minimum concentrations were found 0 013 mM at pH 6 12, 0 021 mM at pH 7 08 and 0 026 mM at pH 7 65 The minimum concentration of procaine was thus considerably lower than that of xylocaine

concentration of the local anesthetic and of the hydrogen ion concentration on the time course

The change in the amplitude of the nerve action potential has previously been used as indicator of the action of the local anesthetic by BENNETT et al (1942) These changes are suitable to indicate the time course of anesthesia in nerve with and without sheath as well as the time course of the restitution of the nerve impulse after anesthesia The action potential amplitude has furthermore been used as a measure for whether the nerve was blocked or not in determining the minimum concentration of the anesthetic To measure conduction velocity the action potential was recorded from two different points on the nerve at known distance form each other A difference in arrival time of the action potential during local anesthesia is then an expression of the effect of the anesthetic on the conduction velocity of the nerve impulse

Sciatic nerves from Hungarian frogs (*Rana esculenta*) were subjected to the anesthetic over a length of 32 mm in a 100 per cent humidified atmosphere of 97 per cent oxygen and 3 per cent carbon dioxide The nerve was stimulated at its distal end with 0 15 msec rectangular pulses the intensity being 2–3 times the strength which evoked an action potential of maximum amplitude In this way the fibers of the A group were stimulated The action potentials just before and just after the anesthetized portion of the nerve were recorded simultaneously on a double beam oscilloscope As anesthesia progressed the amplitude of the post anesthetic action potential diminished while the pre anesthetic action potential gave information as to the condition of the nerve during the anesthesia

By using the action potential amplitude to measure the degree of anesthesia a number of factors must be considered which can affect the amplitude among these factors the dependence of the amplitude on the variation in distance between the recording electrodes was studied and the influence of the degree of stretch of the nerve was investigated During the experiments the amplitude can increase due to an increase in the resistance between the recording electrodes as a result of evaporation of the fluid shunt around the nerve The resistance between the recording electrodes was therefore measured and the amplitude was corrected for any variations

To investigate which portion of the local anesthetic molecule is the anesthetically active component the dissociation of the anesthetic was varied by altering the hydrochloride concentration from 1 to 10 mM and by varying the pH of the solution between 5 5 and 7 8 by means of carbon dioxide bicarbonate and phosphate buffer To ensure the same pH within the nerve as outside it the nerve was maintained in Ringer s solution at the same pH for one hour before the experiment All solutions were isotonic and had the

free diffusion must be ascribed to the effect of subminimal concentrations of the anesthetic and to the fact that the nerve represents an inhomogeneous medium. As to the influence of the nerve sheath the relative time course of anesthesia in nerves without sheath is similar to that of intact nerves but diffusion occurred faster. Assuming proportionality between concentration of free and bound anesthetic substance, binding of the anesthetic to the nerve tissue causes an apparent lag in diffusion without affecting the relative time course.

In single nerve fibers a local anesthetic can be characterized by its minimum concentration. In whole nerves, where a time factor in the action of a local anesthetic is involved, the description of the course of anesthesia is facilitated by the introduction of a "velocity factor". This would allow the time course to be predicted for different concentrations and for nerves of different diameter and could be used to compare a local anesthetic under different conditions and different local anesthetics.

Empirically most of the time course of anesthesia could be predicted from the formula for diffusion into a cylinder and the experimentally determined values for minimum concentration. In the case of xylocaine, for concentrations 3–90 times the minimum concentration, agreement was found between experimental and theoretical time courses until the action potential amplitude was reduced to 15–10 per cent of its value in Ringer's. The "velocity factor" *) determined in this way was $3.0 \times 10^{-7} \pm 0.15 \times 10^{-7}$ cm²/sec. In the case of procaine the agreement between experiment and theory was satisfactory only in the range below 35 per cent of the initial action potential amplitude and at concentrations of 3.5–80 times the minimum concentration. The velocity factor in this range was $2.1 \times 10^{-7} \pm 0.1 \times 10^{-7}$ cm²/sec.

Comparison of nerves with and without sheath

Removal of the sheath resulted in a 2–4 times more rapid anesthesia. As was the case with intact nerves the course of xylocaine anesthesia could be described by diffusion theory when the experimentally found minimum concentrations were used. The "velocity factor" ($8.9 \times 10^{-7} \pm 0.8 \times 10^{-7}$ cm²/sec) was 3 times greater than in nerves with sheath.

With increasing temperature the anesthesia progressed more rapidly in that the time to half amplitude and to blocking of the amplitude due to xylocaine was halved when the temperature was increased from 3–13°C. In the range 13–21°C there was no significant change in anesthetic time.

*) "velocity factor" is used instead of diffusion coefficient to emphasize the empirical nature of the mathematical description.

The pH dependence of the minimum concentration has been considered to indicate that the base component of the anesthetic molecule is not the sole factor which is active during anesthesia (SKOU 1951). In view of the finding that the time course of anesthesia is the same independent of the hydrochloride concentration (and pH) at which a given base concentration was obtained it is suggested that the pH dependence of the minimum concentration indicates an effect of the hydrogen ion concentration on the membrane itself without invalidating the assumption of the base component as the sole anesthetic factor.

The time course of anesthesia as measured by the diminution in action potential amplitude in relation to the theory of free diffusion.

EHRENBERG (1948) has applied the theory of free diffusion to determine diffusion coefficient and minimum concentration from the blocking time at different concentrations of the local anesthetic. I have investigated whether *the time course of the diminution in action potential amplitude* at various concentrations of anesthetic substance can be described in terms of free diffusion of the base component into the nerve. If this were the case one would expect

1) agreement between the experimental and the calculated time course in experiments on nerves with different diameters and using different concentrations of the local anesthetic,

2) that the calculated minimum concentrations be identical with the experimentally determined minimum concentrations, and

3) that the same diffusion coefficient should be determined in experiments with different concentrations of the local anesthetics.

For *xylocaine* a reasonable agreement between theoretical and experimental time course was found until the amplitude was reduced to 15 per cent of its initial value whereafter the experimental time course was slightly slower than the theoretical. On the other hand, the model of free diffusion of the anesthetic substance into the nerve could not be utilized to calculate *minimum concentrations in agreement with those determined* experimentally. The calculated minimum concentrations increased systematically with increasing outer concentration of the anesthetic. Furthermore, identical diffusion coefficients could not be obtained from experiments at different concentrations. For *procaine* there was less agreement between theoretical and experimental time courses than for xylocaine and the calculated minimum concentrations were not identical with those found experimentally.

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That the time course of anesthesia cannot be described solely in terms of

SAMMENFATNING

(Danish Summary)

Formålet med det foreliggende arbejde har været på isolerede frønerver at undersøge det tidsmæssige forløb af en lokalanæsthesi med xylocain og procain og den samtidige virkning på nervens ledningshastighed.

Anæsthesiens tidsmæssige forløb afhænger af forholdet mellem koncentrationen af anæsthesimidlets aktive komponent og den mindste koncentration, der netop kan blokere nerven (minimumskoncentrationen). Minimumskoncentrationen bestemtes eksperimentelt, og det undersøgtes, om anæsthesimidlets uladede basedel var den aktive del af molekylet.

Anæsthesimidlet trænger ind i nerven ved diffusion. Man har derfor villet undersøge, i hvor høj grad anæsthesiens tidsforløb kan forklares ud fra diffusionsloven, og om man, som påstået af EHRENBERG (1948), ud fra modelforestillingen om anæsthesimidlets frie diffusion i nerven kan beregne minimumskoncentrationen og en entydig diffusionskoefficient.

Ud fra det tidsmæssige forløb af nerveimpulsens restitution efter en lokal anæsthesi forsøgte man at afgøre, hvilke faktorer der bestemmer restitutionsforløbet.

Nerveskedens betydning for anæsthesiens tidsforløb undersøgtes ved at sammenligne anæsthesiens tidsforløb i nerver med og uden skede. For at studere virkningen af subminimale koncentrationer af lokalanæsthetika på nervefibermembranen bestemtes nerveimpulsens ledningshastighed under anæsthesiforløbet.

Forsøgsresultaternes betydning for klinisk lokalanæsthesi diskuteres.

Fremgangsmåden

Virkningen af et lokalanæsthetikum er i de fleste tilfælde undersøgt ved psyko-fysiske metoder eller med muskelkontraktionen fra et nerve-muskel præparat som indikator (EHRENBERG 1948, SKOU 1951). Disse fremgangsmåder tillader ikke at følge anæsthesiens tidsforløb med tilstrækkelig nøjagtighed eller at undersøge indflydelsen af anæsthesimidlets koncentration og brintionkoncentration på tidsforløbet.

The restitution of the action potential amplitude when the anesthetic was removed after local anesthesia.

The restitution time of the nerve impulse after anesthesia was measured from the moment when the nerve was just blocked and the anesthetic was replaced by Ringer's until the action potential amplitude had regained 1 and 50 per cent of its pre-anesthetic amplitude Dependent on the base concentration of the local anesthetic the restitution time to 50 per cent amplitude was 2 to 230 times longer than the time to block the nerve to half initial amplitude The restitution time increased with increasing base concentration of anesthetic The minimum concentration for xylocaine and procaine base, determined by extrapolating the restitution time to the concentration which gave instantaneous signs of restitution, was of the same order as that determined directly The restitution time increased to a given limit with the duration of anesthesia At the same base concentration the restitution time after anesthesia was three times less for xylocaine than for procaine owing to the greater minimum concentration and greater "velocity factor" of xylocaine At the same hydrochloride concentration and pH the procaine-treated nerve was restored about 1 5 times more rapidly than that exposed to xylocaine This was due to the fact that xylocaine had the highest base concentration That the time necessary to restore the action potential after removal of the local anesthetic was longer than the blocking time could be explained in part by the slower diffusion out of than into the nerve and in part by the binding of the anesthetic to the nerve tissue which particularly affects the restitution time

The conduction velocity of the nerve impulse under the influence of a local anesthetic.

The conduction velocity progressively diminished over that portion of the nerve exposed to a local anesthetic In the postanesthetic section of the nerve the velocity was unaltered This demonstrates that conduction velocity was not diminished by selective block of the rapidly conducting fibers The diminution in conduction velocity must therefore be due to the effect of the anesthetic in subminimal concentrations This conclusion is supported by the fact that the conduction velocity at the same degree of anesthesia was diminished most by concentrations close to the minimum concentration In this case, of fibers still able to conduct, a greater number are exposed to high subminimal concentrations than with an external concentration which is large as compared with the minimum concentration When the outer base concentration was the same multiple of the minimum concentration the reduction in conduction velocity was the same for xylocaine and for procaine

SAMMENFATNING

(Danish Summary)

Formålet med det foreliggende arbejde har været på isolerede frønerver at undersøge det tidsmæssige forløb af en lokalanæsthesi med xylocain og procain og den samtidige virkning på nervens ledningshastighed

Anæsthesiens tidsmæssige forløb afhænger af forholdet mellem koncentrationen af anæsthesimidlets aktive komponent og den mindste koncentration der netop kan blokere nerven (minimumskoncentrationen) Minimumskoncentrationen bestemtes experimentelt og det undersøgtes om anæsthesimidlets uladede basedel var den aktive del af molekylet

Anæsthesimidlet trænger ind i nerven ved diffusion Man har derfor villet undersøge i hvor høj grad anæsthesiens tidsforløb kan forklares ud fra diffusionsloven og om man, som påstået af EHRENBERG (1918) ud fra modelforestillingen om anæsthesimidlets frie diffusion i nerven kan beregne minimumskoncentrationen og en entydig diffusionskoefficient

Ud fra det tidsmæssige forløb af nerveimpulsens restitution efter en lokal anæsthesi forsøgte man at afgøre hvilke faktorer der bestemmer restitutionsforløbet

Nerveskedens betydning for anæsthesiens tidsforløb undersøgtes ved at sammenligne anæsthesiens tidsforløb i nerver med og uden skede For at studere virkningen af subminimale koncentrationer af lokalanæsthetika på nervefibermembranen bestemtes nerveimpulsens ledningshastighed under anæsthesiforløbet

Forsøgsresultaternes betydning for klinisk lokalanæsthesi diskuteres

Fremgangsmaaden

Virkningen af et lokalanæsthetikum er i de fleste tilfælde undersøgt ved psykofysiske metoder eller med muskelkontraktionen fra et nerve muskel præparat som indikator (EHRENBERG 1918 SKOU 1951) Disse fremgangsmåder tillader ikke at følge anæsthesiens tidsforløb med tilstrækkelig nøjagtighed eller at undersøge indflydelsen af anæsthesimidlets koncentration og brintionkoncentration på tidsforløbet

Ændringen i nerveaktionspotentialets amplitude er tidligere benyttet som indikator for lokalanæsthetikas virkning af BENNETT et al (1942) Disse ændringer er egnede til at udtrykke anæsthesiens tidsforløb i nerver med og uden skede såvel som tidsforløbet af nerveimpulsens restitution efter anæsthesi Aktionspotentialets amplitude anvendes endvidere som mål for, om nerven er blokeret eller ej ved bestemmelse af anæsthesimidlets minimumskoncentration For at måle ledningshastigheden blev aktionspotentialet afledt fra to forskellige steder på nerven med kendt afstand imellem En ændring i aktionspotentialets ankomsttid under en lokalanæsthesi kan betragtes som udtryk for anæsthesimidlets virkning på nerveimpulsens ledningshastighed

Ved forsøgene påvirkes n ischiadicus fra ungarske frøer (*Rana esculenta*) af anæsthesimidlet på et 32 mm langt stykke Nerven stimuleres i den distale ende med rektangulære impulser af 0 15 msek's varighed og en stimulationsstyrke 2–3 gange det maximale aktionspotentials tærskel Herved stimuleres A-gruppens fibre Aktionspotentialet afledes lige før og lige efter det anæstheserede nervestykke i en fugtighedsmættet atmosfære af 97% O_2 og 3% CO_2 og registreres samtidig på en dobbeltstråleoscillograf Anæsthesiens fremadskriden fremgår af reduktionen af det postanæsthetisk afledte aktionspotentials amplitude, mens det præanæsthetiske aktionspotential fungerer som kontrol på nervens tilstand under anæsthesien

Ved at benytte aktionspotentialets amplitude som mål for anæsthesigraden, må der tages hensyn til en række faktorer, der i sig selv kan påvirke amplituden Forsøg blev således gjort over amplitudens afhængighed af variation i afstanden mellem afledningselektroderne og nervens strækningsgrad Under forsøgene kan amplituden stige ved forøgelse af modstanden mellem afledningselektroderne på grund af fordampning af væskeshunten omkring nerven Der foretoges derfor måling af modstanden mellem afledningselektroderne, og amplituden korrigeres for eventuelle variationer

For at undersøge hvilken del af et lokalanæsthetikummolekyle, der er den anæsthetisk aktive komponent må anæsthesimidlets dissociation kunne varieres Dette sker dels ved at ændre hydrokloridkoncentrationen mellem 1 og 10 mM, dels ved variation af opløsningens pH mellem 5 5 og 7 8 ved hjælp af en *kuldioxyd bikarbonatpuffer og en fosfatpuffer* For at sikre, at nervens indre har samme pH som anæsthesimidlet, opbevares nerven i 1 time før forsøget i en specialringeropløsning med den pågældende pH Alle opløsninger har samme koncentration af Ca^{++}, K^{+} og Na^{+} og er isotoniske Dette opnås ved at reducere Ringer-opløsningens koncentration af NaCl til 75 mM, hvorefter der tilsættes anæsthesimiddel eller kolinklorid indtil det osmotiske tryk svarer til 219 m-equiv

Virkningen af et lokalanæsthetikum på aktionspotentialets amplitude

Når nerven påvirkes med et lokalanæsthetikum, ses et øjeblikkeligt fald i aktionspotentialets amplitude. I det senere anæsthesiforløb aftager amplituden langsommere. Den anæsthetiske virkning af xylocain og procainhydroklorid fremskyndes ved stigende koncentration og med forøgelse af pH. Både en forøgelse af hydrokloridkoncentration og af pH betinger en forøgelse af anæsthesimidlets basekoncentration. Forøges basekoncentrationen fire gange med uforandret syrekoncentration forkortes anæsthesiens tidsforløb med mere end 40%. En forøgelse i syrekoncentrationen op til 17 gange påvirker ikke anæsthesiens tidsforløb, hvis basekoncentrationen holdes konstant. Det forekommer derfor sandsynligt, at anæsthesimidlets basedel er den anæsthetisk aktive faktor.

En sammenligning af anæsthesiforløbet med xylocain og procain viser, at anæsthesien ved samme hydrokloridkoncentration og pH, forløber dobbelt så hurtigt med xylocain i forhold til procain. Dette skyldes, at xylocainhydrokloridets dissociationskonstant er 10 gange større end procainhydroklorids.

Til klinisk brug bør man, hvis man ønsker hurtig virkning og mindst mulig toxicitet, anvende en opløsning med så højt pH som muligt fremfor at øge hydrokloridkoncentration.

Minimumskoncentrationen er bestemt som den mindste koncentration der kan blokere en nerve efter mindst 6–8 timers påvirkning. Denne påvirkningstid valgtes, fordi irritabiliteten nedsættes, når en isoleret nerve opbevares i Ringer i 12–24 timer (BOEHNINGHAUS og KOCHMANN 1927). Forsøg med koncentrationer, der ligger lidt over minimumskoncentrationen sandsynliggjorde, at en påvirkningstid på 6–8 timer er tilstrækkelig for at opnå diffusionsligevægt. Overensstemmende med SKOU (1951) finder man, at minimumskoncentrationen for procain bliver større, når anæsthesiopløsningens pH forøges. Det samme er tilfældet for xylocainbasens vedkommende. Minimumskoncentrationen for xylocainbasen er 0.04 mM ved pH 6.0 og 0.11 mM ved pH 7.10. For procainbasen finder man minimumskoncentrationerne 0.013 mM ved pH 6.12, 0.021 mM ved pH 7.08 og 0.026 mM ved pH 7.65. Procains minimumskoncentration er således væsentlig lavere end xylocains. Minimumskoncentrationens pH afhængighed er blevet opfattet som et udtryk for, at anæsthesimidlets basekomponent ikke er den eneste faktor, der er aktiv under anæsthesi (SKOU 1951). I betragtning af, at anæsthesiens tidsforløb er det samme uafhængigt af, ved hvilken hydroklorid koncentration (og pH) en given basekoncentration er opnået, kan man forestille sig, at minimumskoncentrationens pH afhængighed er udtryk for brint

ionkoncentrationens virkning på selve nervemembranen, uden at det ændrer den antagelse, at basekomponenten er den eneste anæsthetiske faktor

Det tidsmæssige forløb af anæsthesien målt ved nedgang i aktionspotentialamplituden og teorien for fri diffusion.

EHRENBERG (1918) har anvendt teorien for fri diffusion til bestemmelse af en diffusionskoefficient og en minimumskoncentration ud fra blokeringstiden med forskellige koncentrationer af lokalanæsthesimidlet I de foreliggende forsøg er det undersøgt, om tidsforløbet af aktionspotentialamplitudens nedgang ved forskellige koncentrationer af anæsthesimidlet kan beskrives ved antagelsen af basekomponentens frie diffusion ind i nerven Hvis dette er tilfældet, skulle man vente

1) overensstemmelse mellem det experimentelle og beregnede tidsforløb i forsøg på nerver med forskellig tykkelse og ved anvendelse af forskellige koncentrationer af anæsthesimidlet,

2) at de beregnede minimumskoncentrationer er identiske med de experimentelt bestemte minimumskoncentrationer, og

3) at den samme diffusionskoefficient bestemmes i forskellige forsøg

For *xylocains* vedkommende finder man rimelig overensstemmelse mellem teoretiske og experimentelle tidsforløb, indtil amplituden er reduceret til 15% af initialværdien, hvorefter de experimentelle tidsforløb er lidt langsommere end de teoretiske På den anden side kan modelforestillingen om anæsthesimidlets frie diffusion i nerven ikke anvendes til at beregne minimumskoncentrationer, der stemmer overens med de direkte experimentelt bestemte De beregnede minimumskoncentrationer vokser systematisk med øget ydre koncentration af anæsthesimidlet Endvidere kan entydige diffusionskoefficienter ikke opnås ved forsøg med forskellige koncentrationer For *procain* er der mindre overensstemmelse mellem det teoretiske og experimentelle tidsforløb end for xylocain, og den beregnede minimumskoncentration er ikke identisk med den experimentelt fundne

At anæsthesiens tidsforløb ikke kan beskrives alene udfra formlen for fri diffusion må tilskrives virkningen af subminimale koncentrationer af anæsthesimidlet og den kendsgerning, at nerven udgør et inhomogent medium Med hensyn til nerveskedens indflydelse svarer anæsthesiens relative tidsforløb i nerver uden skede til det i nerver med skede, men diffusionen foregår hurtigere Hvis der kan forudsættes proportionalitet mellem koncentrationen af frit og bundet anæsthesimiddel, vil binding af anæsthesimidlet til nervevævet medføre en langsommere diffusion uden at påvirke det relative tidsforløb

På enkelte nervefibre kan et lokalanæsthetikum karakteriseres ved dets minimumskoncentration. På hele nerver, hvor der indgår en tidsfaktor i virkningen af et lokalanæsthetikum, lettes beskrivelsen af anæsthesiforløbet ved indførelse af en "hastighedsfaktor". Denne skulle tillade at forudsige tidsforløbet for forskellige koncentrationer og for nerver med forskellig diameter og kunne anvendes til at sammenligne et lokalanæsthetikum under forskellige forhold og sammenligne forskellige lokalanæsthetika.

Empirisk kan en stor del af anæsthesiens tidsforløb forudsiges ud fra formlen for fri diffusion i en cylinder i forbindelse med de experimentelt bestemte værdier for minimumskoncentrationerne.

For xylocains vedkommende finder man for koncentrationer i området 3–90 gange minimumskoncentrationen overensstemmelse mellem det experimentelle og teoretiske forløb, indtil aktionspotentialamplituden er reduceret til 15–10 % af Ringer-værdien. Den tilsvarende hastighedsfaktor *) er $3{,}0 \times 10^{-7} \pm 0{,}15 \times 10^{-7}$ cm²/sek. For procains vedkommende er overensstemmelsen mellem experiment og teori kun god i amplitudeområdet < 35 % i koncentrationsområdet 3,5–80 gange minimumskoncentrationen. Hastighedsfaktoren er i dette område $2{,}1 \times 10^{-7} \pm 0{,}1 \times 10^{-7}$ cm²/sek.

Sammenligning af nerver med og uden skede

Fjernelse af skeden medfører en 2–4 gange hurtigere anæsthesi. Som tilfældet er med intakte nerver, kan xylocainanæsthesiens tidsforløb beskrives ved hjælp af diffusionsteorien, når de experimentelt *fundne* minimumskoncentrationer anvendes. Hastighedsfaktoren ($8{,}9 \times 10^{-7} \pm 0{,}8 \times 10^{-7}$ cm²/sek) var 3 gange større end i nerver med skede.

Med stigende *temperatur* forløber anæsthesien *hurtigere*, idet tiden til halvering og blokering af amplituden ved påvirkning af xylocain halveres ved en temperaturstigning fra 3–13°C. I området 13–21°C er der ingen signifikant forskel i anæsthesitiderne.

Restitution af aktionspotentialets amplitude, når anæsthesimidlet blev fjernet efter en lokalanæsthesi

Nerveimpulsens restitutionstid efter *en anæsthesi* måles fra det øjeblik nerven netop er blokeret og anæsthesimidlet udskiftes med Ringer, til aktionspotentialets amplitude igen er 1 % og 50 % af amplituden i Ringer-forsøget.

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*) Hastighedsfaktor anvendes i stedet for diffusionskoefficient for at fremhæve, at den matematiske beskrivelse er empirisk.

Afhængig af anæsthesimidlets basekoncentration er restitutionstiden til 50% amplitude 2–230 gange længere, end det tager at anæsthesere nerven til det halve af initialamplituden Restitutionstiden foroges med voksende *basekoncentration Minimumskoncentrationen* for xylocain- og procainbase bestemt ved extrapolation af restitutionstiden til den koncentration, der ville give øjeblikkelige *tegn på restitution*, er af samme *størrelsesorden som* de experimentelt fundne *Restitutionstiden foroges indtil en vis grænse med* varigheden af anæsthesien Ved samme basekoncentration er restitutionstiden efter xylocainanæsthesi tre gange mindre end efter procainanæsthesi på grund af xylocains storre minimumskoncentration og større hastighedsfaktor'. Ved samme hydrokloridkoncentration og pH restitueres den procainbehandlede nerve ca 1 5 gange hurtigere end den xylocainbehandlede Dette skyldes, at basekoncentrationen er storst for xylocains vedkommende At tiden til restitution af aktionspotentialet efter fjernelse af lokalanæsthesimidlet er længere end blokeringstiden kan forklares dels ved den langsommere diffusion ud af nerven end ind i nerven, dels ved, at bindingen af *anæsthesimidlet til nervevævet i særlig grad påvirker restitutionstiderne*

Nerveimpulsens ledningshastighed under indflydelse af et lokalanæsthetikum.

Xylocain og procain *nedsætter* ledningshastigheden i det *nervestykke*, der *anæstheseres efterhanden som anæsthesien skrider frem I det post*anæsthetiske stykke af *nerven* er hastigheden imidlertid uforandret Dette viser, at *ledningshastigheden ikke nedsættes ved en selektiv blokering af de* hurtigste fibre Nedsættelsen i ledningshastigheden må derfor skyldes virkning af anæsthesimidlet i ikke blokerende koncentrationer Denne konklusion *støttes af, at ledningshastigheden nedsættes mest af koncentrationer om*kring minimumskoncentrationen I dette tilfælde er der af de fibre, der stadig er i stand til at lede impulser, flere der er påvirket af *høje* subminimale koncentrationer, end ved en yderkoncentration, der er stor i *forhold til minimumskoncentrationen* Når den ydre basekoncentration er samme multiplum af de respektive minimumskoncentrationer, er nedsættelsen i ledningshastighed den samme for xylocain og procain

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CHAPTER 1 | REVIEW OF THE LITERATURE

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Previous investigations of experimental chemically induced pain in humans are few and the results in part contradictory Table 1 lists those authors who have reported — usually as incidental findings — the pain producing effects of various aqueous solutions

Elevation of the osmotic pressure above a certain level, it is reported invariably gives rise to pain According to different authors this level ranges between 2 8 and 5 7 times isotonic sodium chloride solution[2 4 13 29 32 33 37 39 73]

Hypotonic solutions also produce pain if the osmotic pres sure is sufficiently reduced The necessary reduction reportedly varies from 0 63 to 0 23 times isotonic sodium chloride solu tion[2 4 11 13 29 37 73] Distilled water is said to induce severe pain[33 37 73]

Several authors have shown that potassium salts give rise to pain The reported pain producing concentration of K^+ varies however from 134 mN to 0 8 mN the latter value being lower than the potassium concentrat on in plasma[2 4 11 32 33 39 73 77 87]

Fleckenstein[26] stated that certain intermediate metabolites such as pyruvic acid succinic acid and citric acid in neutral isotonic solution gave rise to pain in a concentration of approx imately 10^{2} to 10^{3} g/g

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Table 1 Data on previous investigations of chemically induced skin pain.

AUTHOR	YEAR	MODE OF APPLICATION	PAIN PRODUCING CONCENTRATION						
			ELEVATED OSMOTIC PRESSURE*	REDUCED OSMOTIC PRESSURE*	POTASSIUM ION mN	HYDROGEN ION pH	HISTAMINE G/ML	ACETYL-CHOLINE G/ML	SEROTONIN G/ML
Grutzner[32]	1894	Brushing in wounds	6 6 ×	—	134	1 N HCl	—	—	—
Hacker[33]	1914	Intracutaneous injection	9 1 ×	—	161	0 01 N HCl	—	—	—
Rodhe[73]	1921	Intracutaneous injection	5 7 ×	0 23 ×	134	—	—	—	—
Hecht[37]	1923	Intracutaneous injection	5 7 ×	0 28 ×	—	—	—	—	—
Bommer[11]	1924	Intracutaneous injection	—	0 5 ×	0 8	—	—	—	—
Braun[12]	1925	Intracutaneous injection	2 8 ×	0 63 ×	—	—	—	—	—
Hoff[38]	1926	Intracutaneous injection	5 6 ×	0 34 ×	161	—	—	—	—
Gaza[29]	1926	Intracutaneous injection	2 8 ×	0 28 ×	—	7 1	—	—	—
Rosenthal[76]	1939	Intracutaneous injection	—	—	—	—	0 5 × 10^{8}	2 5 × 10^{-4}	—
Rosenthal[77]	1948	Intracutaneous injection	—	—	13	—	10^{16}	2 × 10^{8}	—
Rosenthal[75]	1950	Intracutaneous injection	—	—	—	—	10^{15}	—	—
Armstrong[8]	1951	Cantharidin blister lesions	3 4 ×	0 34 ×	16	2 5	10^{8}	3 × 10^{6}	—
Armstrong[3]	1952	Cantharidin blister lesions	—	—	—	—	—	—	10^{8}
Armstrong[4]	1953	Cantharidin blister lesions	5 7 ×	0 34 ×	16	3 0	10^{7}	10^{4}	—
Armstrong[5]	1957	Cantharidin blister lesions	—	—	16	—	10^{6}	10^{8}	10^{9}
Skouby[87]	1953	Intracutaneous injection	—	—	40	—	10^{4}	No pain	

Rosenthal et al [74, 75, 76, 7] demonstrated a pain producing effect of **histamine** in a concentration as low as 10^{18} g/g *Skouby*[87] and *Armstrong et al* [2, 4] were able to elicit pain with histamine only when the concentration exceeded 10^{4} to 10^{3} g/g

Armstrong et al [2] induced pain with **acetylcholine** at a concentration of 3 10^{5} whereas *Rosenthal et al*[76, 77] recorded no pain until the concentration reached 0 25 10^{3} to 2 10^{3} g/g and *Skouby*[8] found no pain whatsoever with acetylcholine

Armstrong et al[5] reported that **serotonin** produced pain in a concentration of 10^{9} g/g or higher

Hacker[33] elicited pain with dilute mineral acids *Ga a* & *Brandi*[29] found that buffer solutions of pH 7 2 or lower were pain producing *Armstrong et al* [2, 4] however recorded pain only at pH 3 or lower

The data obtained are thus in part contradictory but above all they are merely qualitative Moreover the reported pain producing concentrations of tested agents vary widely — in the case of histamine for example by as much as 10^{15} times Frequently the reader is left in a state of perplexity as to the significance of various substances in the elicitation of pain

Chemical Methods for Inducing Skin Pain

Varying modes of application have been employed for eliciting pain by chemical means Some authors have placed the test solution directly upon the skin or mucous membrane[1, 51] *This* procedure requires strong solutions and the resulting responses are unreliable

Hence it has been sought instead to apply the test solutions in direct contact with the tissues particularly the naked nerve

terminals. *Armstrong et al.*[2] pricked the skin with a needle through the test solution. *Grutzner*[32] made small skin incisions on the fingers of his subjects, then brushed the test solution into the wounds. *Rosenthal et al.*[76] removed a small piece of the outer epidermal layer with a razor, then dropped the solution on the defect. *Armstrong et al.*[2,4] applied cantharidin to the skin overnight, then opened the resulting blister and dropped the test solution into the skin defect. It was possible to test a number of solutions on the same defect provided the area was rinsed with physiological salt solution and intervals of a minute or so allowed between the tests.

The most common procedure is probably to inject the test solution with fine needles intracutaneously or subcutaneously, or even intramuscularly. *With subcutaneous injections the* pain induced depends upon the point reached by the solution beneath the skin. The pain will be more intense in the vicinity of a nerve and also when the solution is near the epidermis, while deeper in the tissue the same injection will have a less painful effect. Hence the pain responses to solutions tested in this way show a wide range.

Intracutaneous injection seems to be the most frequently employed mode of application.[11,13,26,29,33,37,39,73,75,76,77,87] Even this method has its drawbacks, for instance, the appreciable pain associated with application and the difficulty of *insuring a uniform injection rate and depth.*

Intra-arterial injections have been tried experimentally only in animals.[15,16,23,62] As in all animal experiments, it is here difficult or impossible to estimate the intensity of the pain; it must suffice to record reflex activity, muscle activity, vocalization, etc. There are only occasional instances of intra-arterial injections in humans, but these have yielded useful information.[14,35]

Although intramuscular injections in humans have been

reported[29 58], the examiners have experienced difficulty in grading the pain intensity Pain in muscle tissue is usually diffuse and cannot be readily defined

Methods of Evaluating Pain Intensity

Some investigators have been content to record whether or not pain has occurred, but in general it has been sought to grade the intensity of the pain as one plus, two plus, three plus, etc The numerals 1 to 4 have sometimes been substituted for the plus signs, or in turn have been replaced by definitive terms such as slight, moderate, severe, and very severe[7, 22 41, 43 49].

Hardy, Wolff & Goodell[34] in their extensive experimental investigations of pain induced by heat, introduced the term just noticeable difference", abbreviated jnd They showed that the range between scarcely perceptible and maximal pain comprised 21 jnd intervals Two jnd's were called one dol Trained subjects were able to estimate with some accuracy the pain in dols (pain units) that followed any specific thermal stimulus The relevant scale had a range of zero to ten dols It follows that trained subjects at least are well able to distinguish between varying degrees of pain

Armstrong et al[2 4] employed graphic recording of the pain intensity Their subjects squeezed a rubber balloon connected to a pen the movements of which were recorded on paper

Rosenthal et al[74] undertook statistical analyses to estimate the significance of their observations The experiments conducted by most other authors have not been sufficiently numerous to permit statistical analysis

Recent interest in evaluation of pain for the purpose of investigating the analgetic effects of morphine and similar drugs, has led to the development of reliable statistical methods in conjunction with double blind techniques Although the aim

terminals *Armstrong et al*[2] pricked the skin with a needle *through the test solution* *Grutzner*[32] made small skin incisions on the fingers of his subjects, then brushed the test solution into the wounds *Rosenthal et al*[6] removed *a small piece of* the outer epidermal layer with a razor, then dropped the solution on the defect *Armstrong et al*[2,4] applied cantharidin to the skin overnight, then opened the resulting blister and dropped the test solution into the skin defect It was possible to test a number of solutions on the same defect provided the area was rinsed with physiological salt solution and intervals *of a minute or so allowed between the tests*

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of the present investigation is somewhat different, the problem as well as the methods of evaluation are similar, whether it is a matter of measuring induced pain or of measuring spon taneous pain alleviated by analgesics Investigations of this type have been conducted notably by *Beecher*[7, 88 89], *Houde*[41 83] and *Lasagna*[46 47 48 49] These authors concur on the follow ing general principles for measurement of pain (1) A double blind technique is essential, (2) the subjects should serve as their own controls unless very large numbers of expe rimentees are used, (3) expert statisticians should collaborate in statistical analyses of the observations

The same authors also discuss the question of the propor tionality of the scales when subjective criteria such as slight, moderate and severe pain are employed Alternate use of parametric and non parametric methods of statistical analysis has demonstrated that for comparison of different doses of the *same analgesic, parametric methods (analysis of variance)* are the most precise The authors conclude, accordingly, that these subjective scales are virtually proportional, even though no conclusive evidence thereof can be secured

The few previous investigations into experimental, chemi cally induced pain have not explored the subject with sufficient thoroughness, perhaps chiefly due to lack of uniform methods of pain measurement

In the investigation reported here my aims have been

(1) to devise a *chemical method of producing experimental* skin pain and of measuring its intensity, and

(2) to determine systematically, with the use of that method, the pain producing effects of various substances which occur in the organism and which are thought to be asso ciated with pain

PART I

METHOD FOR INDUCTION OF EXPERIMENTAL SKIN PAIN AND MEASUREMENT OF ITS INTENSITY

CHAPTER 2

EXPERIMENTAL METHOD

Subjects

The experiments were performed on healthy subjects of both sexes ranging in age from 16 to 57 years and averaging thirty six Since the experiments in many instances were very painful, some of the subjects who took part in the first few tests subsequently dropped out This resulted in some degree of selection in that the remaining subjects were apparently more able to endure pain The final group consisted of 20 subjects Their ages varied between 16 and 57, the mean being 39 years

Apparatus

The test solutions were applied by a jet injector (Hygiscient AB Kifa, Stockholm) With this instrument an electric vibration motor operates a piston at very high speed in a cylinder The result is a pump action, which draws solution from the syringe to a nozzle, from which a jet 0 1 mm in diameter is ejected at high velocity and high pressure It perforates the cutis and enters the subcutis The injection procedure itself evokes little or no pain

Trial injections of Evans blue in patients and of India ink in autopsy material showed that the jet entered the cutis and subcutis but did not penetrate the underlying fascia The injection gave rise to an intracutaneous wheal 3 to 6 mm in dia

Intensity of pain

Grade	Units
NONE	0
None to very slight	0 5
VERY SLIGHT	1 0
Very slight to slight	1 5
SLIGHT	2 0
Slight to moderate	2 5
MODERATE	3 0
Moderate to severe	3 5
SEVERE	4 0
Severe to very severe	4 5
VERY SEVERE	5 0

The subjects usually reported grades denoted by full units, the intermediate grades, though less frequently mentioned, were sometimes used to express minor differences in pain and sometimes, perhaps, as a sign of hesitation

Eight different solutions were tested concurrently in each experiment, thus permitting comparison of their pain producing effects Fifteen to 21 subjects — usually 20 — took part in the tests

meter and elevated 1 or 2 mm above the surrounding skin surface In the subcutis was a diffuse conical injection area terminating at the fascia

The duration of pain was recorded in seconds with a stop watch

Procedure

With the aid of this jet injector 0 1 ml test solution was injected in each subject In each run 16 such injections were given consecutively in each subject, eight different solutions being injected first on the outside of one upper arm, then on the contralateral arm The pain caused by one injection was allowed to subside completely before the next injection was given Neither the subjects nor the examiner (the present writer) knew which solutions the syringes contained, since the latter had been prepared for use as follows Bottles containing eight different solutions were "shuffled" and placed in a row, and from them eight numbered syringes were filled in order Not until the experiment had concluded were the solutions identified and annotated in the experimental records The examiner thus knew which eight solutions were being tested but not which one each syringe contained In this way a double blind technique was insured

The subjects for their part estimated both the maximal pain intensity and the duration of pain The estimated intensity was graded as follows

Intensity of pain

Grade	Units
NONE	0
None to very slight	0 5
VERY SLIGHT	1 0
Very slight to slight	1 5
SLIGHT	2 0
Slight to moderate	2 5
MODERATE	3 0
Moderate to severe	3 5
SEVERE	4 0
Severe to very severe	4 5
VERY SEVERE	5 0

The subjects usually reported grades denoted by full units, the intermediate grades, though less frequently mentioned, were sometimes used to express minor differences in pain and sometimes, perhaps, as a sign of hesitation

Eight different solutions were tested concurrently in each experiment, thus permitting comparison of their pain producing effects Fifteen to 21 subjects — usually 20 — took part in the tests

STATISTICAL TREATMENT

Each of the test solutions was injected twice in each subject Since the pain producing effects were evaluated in 15—21 subjects, 30—42 values were obtained for the intensity, and a like number for the duration, of the pain induced by each solution The pain values reported in the following are arith metical means for these 30—42 injections

Insofar as it was desired to compare directly the pain pro ducing effects of two solutions, the average pain response (intensity and duration) of each subject to those solutions was first calculated The difference in the responses to the two solutions was than computed for each subject, after which the 15—21 values thus obtained were used for calculation of the mean (m), the standard deviation (σ) and the standard error of the mean (ε)

$$m = \frac{\Sigma x}{n} \qquad (1)$$

$$\sigma = \sqrt{\frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n - 1}} \qquad (2)$$

$$\varepsilon = \frac{\sigma}{\sqrt{n}} \qquad (3)$$

n = number of observations

x = differences in individual values (of pain intensity or duration)

The difference in pain responses (intensity and duration) to the solutions compared was checked for significance by means of the t test

$$t = \frac{m}{\varepsilon} \tag{4}$$

The p values were taken from conventional tables

In all such comparisons the two solutions had been injected in the same subjects on the same occasions

Regression analyses were undertaken in respect of hyper tonic and hypotonic solutions, solutions with different potas sium ion concentrations, and solutions with different hydrogen ion concentrations These analyses were concerned with the relationship of the various concentrations of pain producing agents to the intensity and duration of pain

A record of experimental tests of varying hydrogen ion con centrations is exemplified below

SOLUTION	PAIN INTENSITY UNITS INJECTION NO		PAIN DURATION SECONDS INJECTION NO	
	1	2	1	2
pH 5 6	1 0	2 0	7	34
pH 3 6	3 0	4 0	36	12
pH 5 1	3 5	2 0	12	8
pH 3 2	4 5	4 5	20	22
pH 4 1	4 0	5 0	22	20
pH 6 2	1 0	1 0	7	5
Sodium acetate	1 0	0 5	8	2
pH 4 6	4 0	4 0	16	19

This record pertains to a subject in whom each solution was injected twice, the two groups of figures for intensity and duration signifying the responses to two different injections.

For evaluation of any correlation that may have existed between the pain-producing effect and the hydrogen ion concentration, regression lines for both intensity and duration were plotted in respect of each subject. The equation for the regression line may be written as:

$$y = b \cdot x + a \qquad (5)$$

where a is the distance of the line from the x axis on the y axis; b is the tangent for the angle of the slope; x is the hydrogen ion concentration (K^+ concentration, osmotic pressure); and y represents the values for pain intensity (units) or pain duration (seconds). The values for b and a were computed from the following formulas:

$$b = \frac{\Sigma xy - \frac{\Sigma x \cdot \Sigma y}{n}}{\Sigma x^2 - \frac{(\Sigma x)^2}{n}} \qquad (6)$$

$$a = \frac{\Sigma y}{n} - b \cdot \frac{\Sigma x}{n} \qquad (7)$$

The arithmethical mean, standard deviation and standard error of the mean for the 20 a and b values were then calculated, in respect of pain intensity and duration, from formulas (1), (2) and (3).

The slope of the average regression line (mean of the b values) was thereafter compared with that of the x axis ($b = 0$), and the difference in slope (mean of the b values) was examined for significance by means of the t test.

A regression analysis was also undertaken in regard to the relation of intensity to duration for different types of pain producing agents, the above formulas (6), (7), being used for computation of the b and a values For checking the significance of the regression a formula differing from the above was used, namely

$$s = \sqrt{\frac{1}{n-2} \cdot \left(u - \frac{v^2}{z}\right)} \quad (8)$$

$$\sigma_b = \frac{s}{\sqrt{z}} \quad (9)$$

The significance was determined by the t test (formula 4), the number of degrees of freedom being $n - 2$

n = number of observations

s = standard deviation about the regression line

x = individual values of pain intensity (units)

y = individual values of pain duration (seconds)

$$u = \Sigma y^2 - \frac{(\Sigma y)^2}{n}$$

$$v = \Sigma xy - \frac{\Sigma x \, \Sigma y}{n}$$

$$z = \Sigma x^2 - \frac{(\Sigma x)^2}{n}$$

In a few instances analysis of covariance was employed to ascertain if the difference in the slopes of two regression lines was significant For this purpose the following formulas were used

This record pertains to a subject in whom each solution was injected twice, the two groups of figures for intensity and duration signifying the responses to two different injections

For evaluation of any correlation that may have existed between the pain producing effect and the hydrogen ion concentration regression lines for both intensity and duration were plotted in respect of each subject The equation for the regression line may be written as

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where a is the distance of the line from the x axis on the y axis, b is the tangent for the angle of the slope, x is the hydrogen ion concentration (K^+ concentration, osmotic pressure), and *y represents the values for pain intensity (units) or pain duration (seconds)* The values for b and a were computed from the following formulas

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The slope of the average regression line (mean of the b values) was thereafter compared with that of the x axis ($b = 0$), and the difference in slope (mean of the b values) was examined for significance by means of the t test

CHAPTER 4

BASAL PAIN ASSOCIATED WITH THE METHOD

In evaluation of the average pain responses to different test solutions, the "basal pain" associated with the method must be taken into account On injection of isotonic sodium chloride solution in 20 subjects, variations occur not only in the individual responses to two injections but also in the average responses of different subjects (figure 1). In the latter instance the varying average responses might be attributable to differing sensitivity to pain, but in the case of two tests on a single subject some other explanation would be more plausible

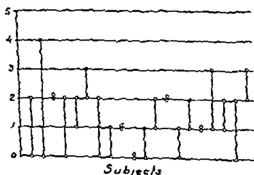


Fig 1 Variation of pain intensity in individual subjects with two similar injections of isotonic sodium chloride solution Each point signifies one pain response, and a vertical line connects the two responses in each subject

$$s_{1+2} = \sqrt{\frac{(n_1 - 2) \cdot s_1^2 + (n_2 - 2) \cdot s_2^2}{n_1 + n_2 - 4}} \qquad (10)$$

$$\varepsilon_{b_1 - b_2} = s_{1+2} \cdot \sqrt{\frac{1}{z_1} + \frac{1}{z_2}} \qquad (11)$$

$$t = \frac{b_1 - b_2}{\varepsilon_{b_1 - b_2}} \qquad (12)$$

The significance wass determined by the t test (formula 12) The number of degrees of freedom was here $n_1 + n_2 - 4$ The symbols have the same signification as before Their subscript numbers denote that they are referable to the two lines which are to be compared

The statistical section of this investigation was planned and executed in consultation with the Statistical Research Group, University of Stockholm

CHAPTER 4

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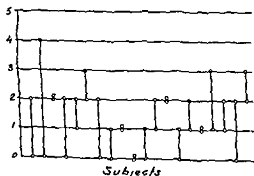
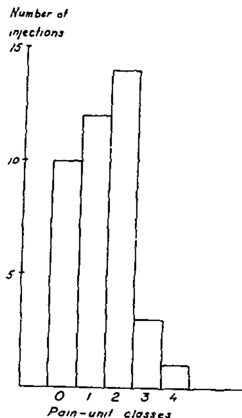


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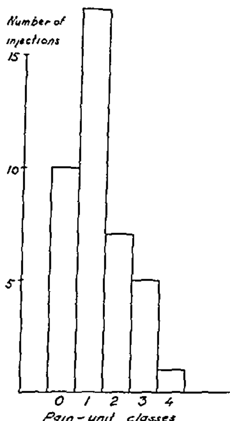
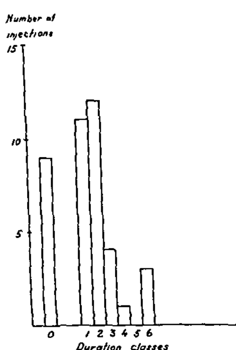


Fig 2 Histogram showing distribution of pain intensity following 40 injections of *isotonic sodium chloride solution in 20 subjects* Average pain intensity for all injections is 1 33 units Pain unit class 0 comprises 0 and 0 5 units, class 1 comprises 1 0 and 1 5 units, etc

Fig 3 Histogram *showing distribution of pain intensity following* 40 injections of isotonic standard salt solution in 20 subjects Average pain intensity for *all* injections is *1* 26 units Pain unit class *0* comprises 0 and 0 5 units, class 1 comprises 1 0 and 1 5 units etc

In many cases the injections were described as quite painless *In some cases they produced very slight or slight pain, and in* three cases moderate pain Only in one of 40 instances did an injection of isotonic sodium chloride solution give rise to severe pain (figure 1) The explanation may be that in general the injection trauma to the tissue is inappreciable and thus causes



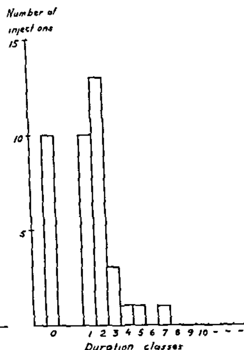
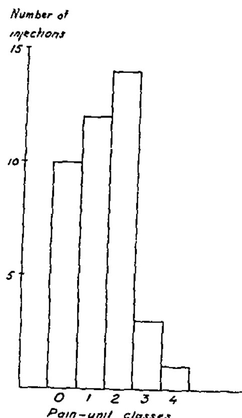


Fig 4 Histogram showing distribution of pain duration following 40 injections of isotonic sodium chloride solution in 20 subjects Average pain duration for all injections is 8 seconds Duration class 0 comprises 0 seconds, class 1 comprises 1—5 seconds, class 2 6—10 seconds, class 3, 11—15 seconds, class 4, 16—20 seconds, etc

Fig 5 Histogram showing distribution of pain duration following 40 injections of isotonic standard salt solution in 20 subjects Average pain duration for all injections is 7 seconds Duration class 0 comprises 0 seconds, class 1 comprises 1—5 seconds, class 2 6—10 seconds, class 3 11—15 seconds, class 4, 16—20 seconds, etc

no pain, while in other cases the jet of fluid is more traumatizing and gives rise to a certain amount of pain due to the tissue injury

On injection of solutions which are pain producing per se it is essential, therefore, to take into account the basal pain associated with the method, i e, the pain caused by the injec



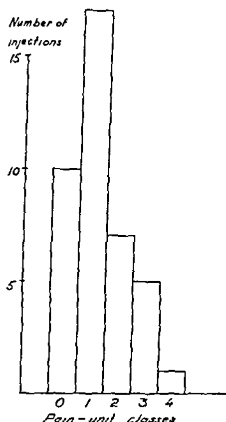


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In many cases the injections were described as quite painless In some cases they produced very slight or slight pain, and in three cases moderate pain Only in one of 40 instances did an injection of isotonic sodium chloride solution give rise to severe pain (figure 1) The explanation may be that in general the injection trauma to the tissue is inappreciable and thus causes

CHAPTER 5

STANDARD DEVIATION AND REPRODUCIBILITY OF THE METHOD

To estimate the standard deviation of the method, the following run of experiments was conducted

Each of 20 subjects received 16 injections, the same solution being used throughout — a neutral isotonic potassium sodium

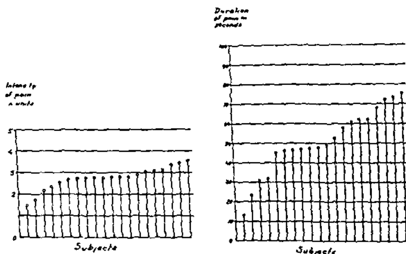


Fig 6 Variation of average pain intensity in 20 subjects who received a total of 320 injections of the same solution containing 77 7 mN potassium Each point represents the mean of 16 injections in one subject The responses are grouped according to their intensity

Fig 7 Variation of average pain duration in 20 subjects who received a total of 320 injections of the same solution containing 77 7 mN potassium Each point represents the mean of 16 injections in one subject The responses are grouped according to their duration

tion procedure itself Since *isotonic sodium chloride solution* normally causes no pain in wounds or on subcutaneous injection, the basal pain must be regarded as an effect of the injection itself and not of the solution per se

Statistically significant pain above the basal level would thus indicate that the tested solution was, in itself, pain producing

Isotonic *sodium chloride solution, in contrast to extracellular* fluid, contains only sodium and chloride ions and hence could conceivably have a slight pain producing effect To check this possibility I prepared an isotonic salt solution containing the ordinary body cations as chlorides and in the same concentration as that occurring in plasma It was termed "standard salt solution" (see page 32) In one run of experiments I tested it along with the *isotonic sodium chloride solution* in the same subjects The average pain responses as well as the distributions of observations were virtually identical for the two solutions

	NUMBER OF SUBJECTS	PAIN INTENSITY UNITS	PAIN DURATION SECONDS
Sodium chloride solution	20	1 33	8
Standard salt solution	20	1 26	7

Cf figures 2, 3, 4 and 5

In some runs neither of these two solutions was used, the pain producing effects of test solutions were instead evaluated in comparison with e g isotonic sodium *bicarbonate or isotonic* sodium acetate solution Each of these two solutions as will be reported later on, shows the same low average pain response as isotonic sodium chloride solution

tested on 15—21 subjects constitutes a further substantial reduction of the experimental error

In any comparison of the pain producing effects of two solutions the methodologic error, however, is automatically taken into account and the analysis shows whether a difference is statistically significant or not

Although the individual pain reaction to a given chemical stimulus varies considerably, as do the reactions of different individuals to the same painful stimulus, this method affords both numerical data on the pain intensity and a possibility of determining whether differences exist between test solutions provided enough injections and subjects are used

The reproducibility of the method is evident from its standard deviation and also from a comparison of the pain intensity and duration values obtained on testing of a given solution on different occasions Isotonic sodium chloride solution and standard salt solution, which both represent the basal pain of the method, were tested on four different occasions largely on the same subjects The following values emerged

DATE	SOLUTION	NUMBER OF SUBJECTS COMMON TO ALL EXPERIMENTS	PAIN DURATION SECONDS	PAIN INTENSITY UNITS
Febr./58	Standard salt	18	1 33	12
April/58	— —	19	1 23	9
Febr./59	— „ —	20	1 26	7
Febr./59	Sodium chloride	20	1.33	8

It will be seen that the various determinations are in close accord

The distribution of values in the series of 320 injections of the same solution (77 7 mN K⁺) in 16 subjects was also studied This distribution is illustrated in figures 8 and 9

chloride solution containing 77 7 mN K^+ Such solution in duces pain of moderate intensity

The average pain response to the 16 injections was calculat ed for each subject In the 20 subjects the mean intensity varied between 1 47 and 3 56 units, and the mean duration between 14 and 75 seconds (figures 6 and 7)

The variation in the values for any single subject — dis regarding the varying sensitivity to pain of different subjects — can be computed statistically from the following formula

$$\sigma = \sqrt{\frac{\Sigma ssq}{N - r}} \qquad (13)$$

σ = standard deviation of the method

ssq = sum of squares of deviations from the mean of *one subject*

N = total number of observations (in this run, 320)

r = number of subjects (in this run, 20)

Σ = sum of all subjects

Calculation by the above formula gives the following values of the standard deviation for each single observation

$\sigma_{intensity}$ = 0 64 units

$\sigma_{duration}$ = 18 seconds

The average pain intensity in all experiments on all subjects was 2 75 units, and the average pain duration 51 seconds The coefficient of variation for a single observation was 23 per cent for intensity and 33 per cent for duration of pain For a biologic method, these values must be considered satisfactory

Since the subjects always received two injections of each solution, the coefficient of variation for each subject amounts to 16 per cent for intensity and 23 per cent for duration of pain The fact that the pain producing effects of solutions were

tested on 15—21 subjects constitutes a further substantial reduction of the experimental error

In any comparison of the pain producing effects of two solutions the methodologic error, however, is automatically taken into account and the analysis shows whether a difference is statistically significant or not

Although the individual pain reaction to a given chemical stimulus varies considerably, as do the reactions of different individuals to the same painful stimulus, this method affords both numerical data on the pain intensity and a possibility of determining whether differences exist between test solutions provided enough injections and subjects are used

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DATE	SOLUTION	NUMBER OF SUBJECTS COMMON TO ALL EXPERIMENTS	PAIN DURATION SECONDS	PAIN INTENSITY UNITS
Febr./58	Standard salt	18	1.33	12
April/58	— —	19	1 23	9
Febr./59	— —	20	1 26	7
Febr./59	Sodium chloride	20	1.33	8

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The average pain response to the 16 injections was calculated for each subject In the 20 subjects the mean intensity varied between 1 47 and 3 56 units, and the mean duration between 14 and 75 seconds (figures 6 and 7)

The variation in the values for any single subject — disregarding the varying sensitivity to pain of different subjects — can be computed statistically from the following formula

$$\sigma = \sqrt{\frac{\Sigma ssq}{N-r}} \qquad (13)$$

σ = *standard deviation of the method*
ssq = sum of squares of deviations from the mean of one subject
N = *total number of observations (in this run, 320)*
r = number of subjects (in this run, 20)
Σ = sum of all subjects

Calculation by the above formula gives the following values of the standard deviation for each single observation

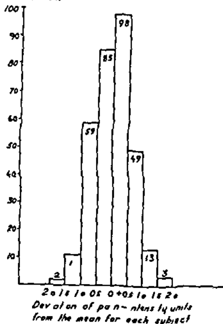
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PART II

PAIN-PRODUCING EFFECTS OF THE TEST SOLUTIONS



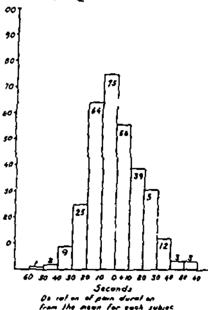


Fig 8 Histogram showing distribution of pain intensity in 20 subjects who received a total of 320 injections of the same solution containing 77 7 mN potassium The classes are based on the mean of each subject thus eliminating the variation of the mean for the individual subjects Class range is 0 5 pain intensity units

Fig 9 Histogram showing distribution of pain duration in 20 subjects who received a total of 320 injections of the same solution containing 77 7 mN potassium The classes are based on the mean of each subject, thus eliminating the variation of the mean for the individual subjects Class range is 10 seconds

In this investigation the deviation from the mean for each subject was calculated in classes, each class range being half a unit for intensity and ten seconds for duration The deviations from the mean for each subject were then totalled in the respective classes, thus securing the total number of observations in each class

The observations both for intensity and for duration of pain showed a normal distribution

CHAPTER 6 | TEST SOLUTIONS

The solutions employed in this investigation were all aqueous and were prepared at the St Gorans Sjukhus pharmacy. Their osmotic pressures were ascertained by determining the reduction of the freezing point, using a method devised by *Bergstrom*[8]. The pH was determined by a pH meter (model pH M 4, Radiometer, Copenhagen). Acetylcholine and serotonin were prepared under aseptic precautions Other solutions were sterilized in an autoclave (110° C for 30 minutes).

Solutions with the following composition were used·

Solutions with Varying Osmotic Pressures

SOLUTION	SODIUM CHLORIDE mM	OSMOTIC PRESSURE MILLIOSMOL/L	pH
1 × isotonic	150	302	7.1
2 × „	300	610	7.1
3 × „	450	902	7.1
4 × „	600	1207	7.2
5 × „	750	1505	7.3
6 × „	900	1802	7 3
0 8 × isotonic	120	245	7.2
0 6 × „	90	180	7.2
0 4 × „	60	119	7.1
0.2 × „	30	59	7.1
0 × „	0	2	7.2

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5 × "	750	1505	7.3
6 × "	900	1802	7.3
0 8 × isotonic	120	245	7.2
0 6 × "	90	180	7.2
0 4 × "	60	119	7.1
0.2 × "	30	59	7.1
0 × "	0	2	7.2

Standard Salt Solution

To obtain a solution differing as little as possible from plasma in regard to mineral salts, the chlorides of sodium, potassium, calcium and magnesium were dissolved in distilled water in the following proportions: sodium 154, potassium 5, calcium 5 and magnesium 3 mN. This plasma concentration of cations had been reported by *Peters*[67]. The solution had an osmotic pressure of 316 milliosmol/1 and a pH of 7 3

Solutions with Varying Potassium Concentrations

These solutions were prepared by mixing the above standard salt solution (osmotic pressure 316 milliosmol/1) with 161 mN potassium chloride solution (osmotic pressure 318 milliosmol/1), the resulting solutions having the following potassium content

POTASSIUM ION CONCENTRATION mN	pH
5 0	7.0
18 4	7 0
31.8	6 9
45 2	7 0
58 6	7 1
72.0	7 0
85 4	7 0
98 8	6 8
77.7	6 9 (Method test solution)

Isotonic Inorganic Salt Solutions

These consisted of the following salts dissolved in distilled water.

SALT	CONCENTRATION mM	OSMOTIC PRESSURE MILLIOSMOL/L	pH
Sodium chloride	150	302	7.1
Magnesium chloride	265	327	7 5
Calcium chloride	226	310	5 3
Ammonium chloride	150	312	6 7
Sodium sulfate	132	325	7 4
Sodium bicarbonate	175	315	8 3
Sodium orthophosphate (mono — H)	130	298	7 4

Isotonic Solutions of Sodium Salts with Organic Acids

For these solutions the undermentioned salts were dissolved in distilled water

SALT	CONCENTRATION mM	OSMOTIC PRESSURE MILLIOSMOL/L	pH
Sodium citrate	108	310	8 8
Sodium lactate	218	285	7 4
Sodium acetate	151	295	8 5
Sodium succinate	101	283	8 1
Sodium pyruvate	153	239	8 4
Sodium acetate and Sodium chloride	100 51	305	8 0

The last solution had the same acetate ion content as the acetate buffers with which it was compared

Solutions of Organic Compounds

Creatine

Creatine 99 mM and sodium chloride 125 mM were dissolved in distilled water The osmotic pressure was 302 *milliosmol/l* and the pH 7 4 The water solubility of creatine does not suffice for an isotonic solution of creatine alone

Standard Salt Solution

To obtain a solution differing as little as possible from plasma in regard to mineral salts, the chlorides of sodium, potassium, calcium and magnesium were dissolved in distilled water in the following proportions sodium 154, potassium 5, calcium 5 and magnesium 3 mN This plasma concentration of cations had been reported by *Peters*[67] The solution had an osmotic pressure of 316 milliosmol/l and a pH of 7 3

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POTASSIUM ION CONCENTRATION mN	pH
5 0	7 0
18 4	7 0
31 8	6 9
45 2	7 0
58 6	7 1
72 0	7 0
85 4	7 0
98 8	6 8
77 7	6 9 (Method test solution)

Isotonic Inorganic Salt Solutions

These consisted of the following salts dissolved in distilled water

Measured pH 3 2 97 5 ml 0 1 N acetic acid — 2 5 ml 0 1 N sodium acetate — 0 36 g sodium chloride O motic pressure 390 milliosmol/l
Measured pH 5 1 29 0 ml 0 1 N acetic acid — 71 0 ml 0 1 N sodium acetate — 0 19 g sodium chloride Osmotic pressure 341 milliosmol/l
Measured pH 7 2 29 6 ml 0 1 N NaOH — 50 ml 0 1 M KH_2PO_4 — 20 4 ml distilled water — 0 54 g sodium chloride Osmotic pressure 220 milliosmol/l

Series with acetate buffers and pH from 3 2 to 6.2

MEASURED pH	0 1 N ACETIC ACID ML	0 1 N SODIUM ACETATE ML	SODIUM CHLORIDE G	OSMOTIC PRESSURE MILLIOSMOL/L
3 2	97 5	2 5	0 36	390
3 6	92 5	7 5	0 31	330
4 1	80 0	20 0	0 29	333
4 6	57 5	42 5	0 26	341
5 1	29 0	71 0	0 19	341
5 6	12 0	88 0	0 15	352
6 2	4 0	96 0	0 13	349

Series with ammonia buffers and pH from 7 6 to 10 6

MEASURED pH	0 1 N H NCl ML	0 1 N H N ML	SODIUM CHLORIDE G	OSMOTIC PRESSURE MILLIOSMOL/L
7 6	98 0	2 0	0 14	364
8 1	93 5	6 5	0 16	339
8 6	81 5	18 5	0 17	356
9 1	58 0	42 0	0 18	320
9 6	31 0	69 0	0 26	328
10 1	12 5	87 5	0 26	324
10 6	4 5	95 5	0 27	326

Creatinine

Creatinine 272 mM and sodium chloride 21 mM were dissolved in distilled water The osmotic pressure was 298 milliosmol/l and the pH 6 5 The water solubility of creatinine is not sufficient for preparation of an isotonic solution with that substance alone

Acetylcholine

Acetylcholine chloride was dissolved in the aforementioned standard salt solution in concentrations of 5 1 mM or 10^{3} g/ml (pH 7 0) and 0 5 mM or 10^{4} g/ml (pH 7 0) The solutions were prepared under aseptic precautions immediately prior to the injections

Serotonin

Serotonin was dissolved in the standard salt solution in concentrations of 5 7 mM or 10^{3} g/ml (pH 7 5), 0 6 mM or 10^{4} g/ml (pH 7 5) and 0 06 mM or 10^{5} g/ml (pH 7 6) The solutions were prepared under aseptic precautions immediately before the injections

Histamine

Histamine chloride was dissolved in the standard salt solution in concentrations of *5 6 mM or 10^{3} g/ml (pH 5 8), 0 6 mM* or 10^{4} g/ml (pH 6 0) 0 06 mM or 10^{5} g/ml (pH 6 5) and *0 006 mM or 10^{6} g/ml* (pH 7 1)

Solutions with Varying Hydrogen Ion Concentrations

Series with different buffers and pH from 1 1 to 7 2

Measured **pH 1 1** *48 5 ml 0 2* N HCl — 25 ml 0 2 N KCl — 26 5 ml distilled water Osmotic pressure 256 milliosmol/l

Measured pH 3 2 97 5 ml 0 1 N acetic acid — 2 5 ml 0 1 N sodium acetate — 0 36 g sodium chloride O motic pressure 390 milliosmol/l
Measured pH 5 1 29 0 ml 0 1 N acetic acid — 71 0 ml 0 1 N sodium acetate — 0 19 g sodium chloride Osmotic pressure 341 milliosmol/l
Measured pH 7 2 29 6 ml 0 1 N NaOH — 50 ml 0 1 M KH_2PO_4 — 20 4 ml distilled water — 0 54 g sodium chloride O motic pressure 220 milliosmol/l

Series with acetate buffers and pH from 3 2 to 6 2

MEASURED pH	0 1 N ACETIC ACID ML	0 1 N SODIUM ACETATE ML	SODIUM CHLORIDE G	OSMOTIC PRESSURE MILLIOSMOL/L
3 2	97 5	2 5	0 36	390
3 6	92 5	7 5	0 31	330
4 1	80 0	20 0	0 29	333
4 6	57 5	42 5	0 26	341
5 1	29 0	71 0	0 19	341
5 6	12 0	88 0	0 15	352
6 2	4 0	96 0	0 13	349

Series with ammonia buffers and pH from 7 6 to 10 6

MEASURED pH	0 1 N H NCl Ml	0 1 N H N Ml	SODIUM CHLORIDE G	OSMOTIC PRESSURE MILLIOSMOL/L
7 6	98 0	2 0	0 14	364
8 1	93 5	6 5	0 16	339
8 6	81 5	18 5	0 17	336
9 1	58 0	42 0	0 18	320
9 6	31 0	69 0	0.26	328
10 1	12 5	87 5	0 26	324
10 6	4 5	95 5	0 27	326

Substances conforming with the requirements of the Swedish Pharmacopoeia Edition XI

Acetic acid
Ammonium chloride
Ammonium hydroxide
Calcium chloride ($CaCl_2 \cdot 2 H_2O$)
Distilled water
Histamine chloride (di chloride)
Hydrochloric acid
Lactic acid
Potassium chloride
Potassium orthophosphate (K_2HPO_4)
Sodium acetate ($NaC_2H_3O_2$)
Sodium bicarbonate
Sodium chloride
Sodium citrate ($Na_3C_6H_5O_7 \cdot 2 H_2O$)
Sodium hydroxide
Sodium sulfate ($Na_2SO_4 \cdot 10 H_2O$)

Substances obtained from E. Merck, Darmstadt

Creatine
Creatinine
Magnesium chloride
Sodium orthophosphate (Na_2HPO_4)
Sodium pyruvate ($NaC_3H_3O_3$)
Sodium succinate ($Na_2C_4H_4O_8 \cdot 6 H_2O$)

Substances obtained from F. Hoffman La Roche & Co. A. G., Basle

Acetylcholine chloride

Substances obtained from Sandoz A.G., Basle

Serotonin (5 hydroxytryptamine)

Sodium lactate ($NaC_3H_5O_3$) was prepared from lactic acid and sodium hydroxide

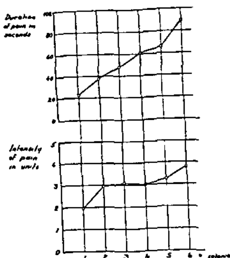
Hypertonic Solutions

For determination of the pain producing effect of an increased osmotic pressure, six neutral aqueous solutions of sodium chloride were tested in one run of experiments The osmotic pressures of these solutions ranged from isotonic to six times the osmotic pressure of plasma All six of them were injected on the same occasion, each subject receiving two injections of each solution The experiment comprised 15 subjects The reported average intensities and durations are the arithmetical means of 30 injections

The results are set forth in table 2 and figure 10

Table 2 Average pain responses to neutral hypertonic sodium chloride solutions tested on 15 subjects Each solution injected 30 times All solutions tested concurrently

SOLUTION	CONCENTRATION OF NaCl mM	OSMOTIC PRESSURE MILLIOSMOL/L	PAIN INTENSITY UNITS	PAIN DURATION SECONDS
1 × isotonic	150	302	1 98	23
2 × "	300	610	2 98	38
3 × "	450	902	3 10	48
4 × "	600	1207	3 02	61
5 × "	750	1505	3.33	66
6 × "	900	1802	3 80	89



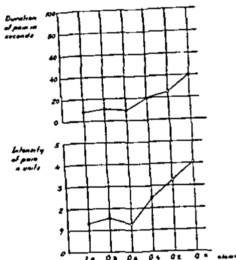


Fig 10 Diagram showing relation of pain — measured as intensity and duration — to elevated osmotic pressure Each point on the curves represents the mean of 30 injections in 15 subjects The test solutions were of sodium chloride with an osmotic pressure ranging from iso tonic to six times that of the body

Fig 11 Diagram showing relation of pain — measured as intensity and duration — to reduced osmotic pressure Each point on the curves represents the mean of 40 injections in 20 subjects The test solutions were of sodium chloride with an osmotic pressure ranging from zero to isotonic with that of the body

It will be seen that elevation of the osmotic pressure above the normal gave rise to pain, which increased proportionately with the osmotic pressure Analysis revealed a statistically significant correlation between osmotic pressure and both in tensity and duration of pain

The following *b* values were obtained for the regression lines for the correlation between increased osmotic pressure and pain

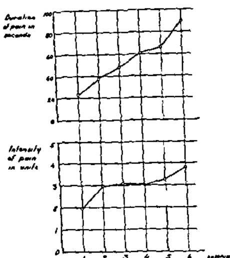
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2 × ,,	300	610	2 98	38
3 ×	450	902	3 10	48
4 × ,	600	1207	3 02	61
5 × ,,	750	1505	3 33	66
6 × ,	900	1802	3 80	89



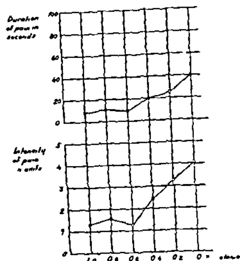


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Fig 11 Diagram showing relation of pain — measured as intensity and duration — to reduced osmotic pressure Each point on the curves represents the mean of 40 injections in 20 subjects The test solutions were of sodium chloride with an osmotic pressure ranging from zero to isotonic with that of the body

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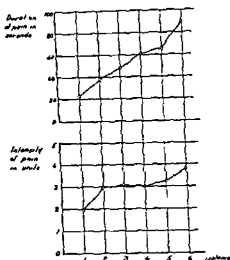
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3 × ,,	450	902	3 10	48
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5 × ,,	750	1505	3 33	66
6 × ,,	900	1802	3 80	89



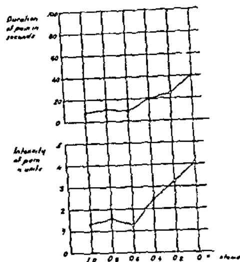


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It will be seen that elevation of the osmotic pressure above the normal gave rise to pain, which increased proportionately with the osmotic pressure Analysis revealed a statistically significant correlation between osmotic pressure and both in tensity and duration of pain

The following *b* values were obtained for the regression lines for the correlation between increased osmotic pressure and pain

Pain intensity $b = 0\ 29 \mp 0\ 03$ $(p < 0\ 001)$
Pain duration $b = 12\ 2 \mp 2\ 1$ $(p < 0\ 001)$

The pain was of unusually long duration for hypertonic solutions, and for the six times isotonic sodium chloride solu tion it averaged 89 seconds — the longest mean duration ob served in this investigation

Hypotonic Solutions

With the aim of studying the pain producing effect of a re duced osmotic pressure, six neutral aqueous solutions of so dium chloride were tested Their osmotic pressures ranged from isotonic with plasma to the value for distilled water The various solutions were tested concurrently on 20 subjects, each solution being injected 40 times The results are presented in table 3 and figure 11

Table 3 Average pain response to neutral hypotonic sodium chloride solutions tested on 20 subjects Each solution injected 40 times All solutions tested concurrently

SOLUTION	CONCENTRA TION OF NaCl mM	OSMOTIC PRESSURE MILLIOSMOL/L	PAIN INTENSITY UNITS	PAIN DURATION SECONDS
1 × isotonic	150	302	1 33	8
0 8 × ,	120	245	1 58	11
0 6 × "	90	180	1 28	9
0 4 × ,	60	119	2 45	20
0 2 × "	30	59	3 29	25
0 × ,	0	2	4 05	40

It will be seen that a moderate decrease of the osmotic pressure did not induce pain Not until the osmotic pressure

fell to 119 milliosmol/l or lower did the solutions cause pain, which increased with decreasing osmotic pressure and was, with distilled water, almost as severe as any recorded in this investigation

Reduced osmotic pressure showed, on analysis, a highly significant correlation to both intensity and duration of pain

As regards the relationship of decreased osmotic pressure to pain, the following *b* values were obtained for the regression lines

Pain intensity	$b = 0.56 \mp 0.03$ $(p < 0.001)$
Pain duration	$b = 6.1 \mp 0.6$ $(p < 0.001)$

Relationship of Pain Intensity to Pain Duration

A study of figures 10 and 11 reveals that the intensity and duration of pain showed largely parallel variations with changes in degree of the painful stimulus Calculation of the regression lines for the correlation between intensity and duration discloses not only that there is a highly significant regression for both hypertonic and hypotonic solutions $(p < 0.001)$ but also that the slope of the regression line differs for the two types of solutions (figure 16, page 60), the difference being highly significant $(p < 0.001)$

INORGANIC IONS

Isotonic neutral solutions of the inorganic ions commonly oc curring in the blood were tested in the form of sodium salts or chlorides Since the solutions were not tested concurrently but in different runs of experiments, they cannot be directly compared The pain producing effects are set forth in table 4, which shows that, for each solution, the average pain intensity was very slight to slight and the duration short

Table 4 Average pain responses to solutions of various in organic ions tested on 20 subjects, though not all concurrently Each solution injected 40 times The solutions were isotonic and their compositions are reported on page 32 Those tested concurrently have identical serial numbers

SALT	SERIES NO	PAIN INTENSITY UNITS	PAIN DURATION SECONDS
$NaCl$	7	1 33	8
$MgCl_2$	7	1 48	11
$CaCl_2$	8	1 90	11
Na_2SO_4	8	1 90	15
H_4NCl	9	2 05	24
$NaHCO_3$	9	1 46	14
Na_2HPO_4	9	1 46	11

Only magnesium chloride solution was tested concurrently with isotonic sodium chloride solution (basal pain of the method). No difference was found in the pain producing effects of these solutions.

Since the other solutions were not tested concurrently with isotonic sodium chloride or standard salt solution, no accurate estimate can be made of their pain producing effects. The pain responses to sodium bicarbonate and sodium phosphate were numerically at the same level as isotonic sodium chloride solution, and the same subjects took part in the two experiments, hence, those two solutions as well may be considered equivalent to the basal pain level.

Comparison of sodium acetate (practically equivalent to the basal pain) with calcium chloride and with sodium sulphate revealed no significant difference. Comparison of sodium bicarbonate and ammonium chloride showed an almost significant difference with respect to pain intensity ($0.01 < p < 0.05$).

Potassium Ion

Previous investigators have attributed marked pain inducing properties to this ion, in contrast to the *aforementioned* ions. This led me to test K^+ in eight different concentrations ranging from 5.0 mN — which is the normal concentration in plasma — to 98.8 mN. The test solutions were neutral aqueous ones, the potassium concentration being varied by admixture of isotonic potassium chloride solution with standard salt solution in varying proportions. The pain producing effects of these solutions are evident from table 5 and figure 12, which show that the pain response rose in direct relation to increasing potassium ion concentration.

Regression analysis disclosed a highly significant correlation between concentration and both intensity and duration of pain ($p < 0.001$).

CHAPTER 8

INORGANIC IONS

*Isotonic neutral solutions of the inorganic ions commonly oc*curring in the blood were tested in the form of sodium salts or chlorides Since the solutions were not tested concurrently but in different runs of experiments, they cannot be directly compared The pain producing effects are set forth in table 4, which shows that, for each solution, the average pain intensity was very slight to slight and the duration short

Table 4 Average pain responses to solutions of various inorganic ions tested on 20 subjects, though not all concurrently Each solution injected 40 times The solutions were isotonic *and their compositions are reported on page 32* Those tested concurrently have identical serial numbers

SALT	SERIES NO	PAIN INTENSITY UNITS	PAIN DURATION SECONDS
NaCl	7	1 33	8
$MgCl_2$	7	*1 48*	11
$CaCl_2$	8	1 90	11
Na_2SO_4	8	1 90	15
H_4NCl	9	2 05	24
$NaHCO_3$	9	1 46	14
Na_2HPO_4	9	1 46	11

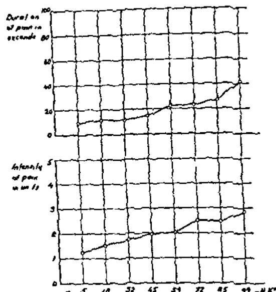


Fig 12 Diagram showing relation of pain — measured as intensity and duration — to the potassium ion concentration Each point on the curves represents the mean of 40 injections in 20 subjects The test solutions were neutral and isotonic, with a potassium ion content ranging from 5 to 99 mN

was still less than moderate, even though the potassium content exceeded that in plasma by approximately 20 times

There was here, just as in the case of solutions with differing osmotic pressures, a correlation between intensity and duration of pain Calculation of the regression line for this correlation showed a highly significant regression ($p < 0.001$). See also figure 16, page 60

Table 5 Average pain responses to neutral isotonic solutions in which the potassium content ranged from 5.0 to 98.8 mN. Each solution tested on 20 subjects and injected 40 times. All solutions tested concurrently

POTASSIUM ION CONCENTRATION mM	PAIN INTENSITY UNITS	PAIN DURATION SECONDS
5.0	1.23	9
18.4	1.54	12
31.8	1.78	12
45.2	1.95	17
58.6	2.05	24
72.0	2.53	25
85.4	2.49	27
98.8	2.85	41

The following *b* values were obtained for the regression lines in respect of the correlation between elevated potassium *ion concentration and pain:*

Pain intensity $b = 0.22 \mp 0.02$ $(p < 0.001)$
Pain duration $b = 4.1 \mp 0.8$ $(p < 0.001)$

Comparison of standard salt solution (K^+ content 5 mN) and the solution with a K^+ *content of 18.4 mN showed no* significant difference. On the other hand, a solution containing 31.8 mN potassium, when compared with standard salt solution, showed a significant difference for pain *intensity* $(0.001 < p < 0.01)$ and an almost significant difference for pain duration $(0.01 < p < 0.05)$. At a potassium concentration of 45.2 mN the difference was significant in respect to both the *intensity and the duration of pain*. However, the pain induced was slight and its duration short. For the solution with the highest potassium concentration (98.8 mN) the average pain

The pain responses to sodium succinate and sodium pyruvate appear to have been somewhat greater than the others Those solutions were tested concurrently with isotonic sodium chlor ide, which in this experiment gave rise to an average pain intensity of 1 94 units Comparison with that solution revealed no significant difference This particular experiment com prised subjects with high average pain responses

Since the other solutions were not tested concurrently with isotonic sodium chloride or standard salt solution their pain producing effects cannot be estimated with any accuracy The pain responses to sodium acetate and sodium lactate were numerically very low, however, hence it seems unlikely that these solutions give rise to any significant pain

In one experiment creatinine, creatine and sodium citrate were tested concurrently No solution representing the basal pain of the method was included in this run The pain respon ses to creatinine and creatine were, numerically, exceptionally low and differed significantly from the response to sodium citrate ($p < 0.001$ both for intensity and duration) Compared with creatinine, therefore, sodium citrate had a pain produc ing effect

ORGANIC METABOLITES

Pain inducing properties have, during the course of the years, been ascribed to various organic metabolites. I have investigated creatinine, creatine, sodium citrate, sodium lactate, sodium acetate, sodium succinate, and sodium pyruvate in isotonic concentrations and as neutral salts. The results are set forth in table 6, from which it is evident that all of these solutions elicited pain responses of both low intensity and short duration.

Table 6 Average pain response to solutions of various organic metabolites. The solutions were isotonic and their compositions are reported on page 33. Those tested concurrently have identical serial numbers. Each subject received two injections.

METABOLITE	NUMBER OF SUBJECTS	SERIES NO	PAIN INTENSITY UNITS	PAIN DURATION SECONDS
Creatinine	20	10	1.14	6
Creatine	20	10	0.96	8
Sodium citrate	20	10	1.94	32
Sodium lactate	20	8	1.68	14
Sodium acetate	20	8	1.53	14
Sodium succinate	15	3	2.40	35
Sodium pyruvate	15	3	2.30	31

The pain responses to sodium succinate and sodium pyruvate appear to have been somewhat greater than the others Those solutions were tested concurrently with isotonic sodium chlor ide, which in this experiment gave rise to an average pain intensity of 1 94 units Comparison with that solution revealed no significant difference This particular experiment com prised subjects with high average pain responses

Since the other solutions were not tested concurrently with isotonic sodium chloride or standard salt solution their pain producing effects cannot be estimated with any accuracy The pain responses to sodium acetate and sodium lactate were numerically very low, however, hence it seems unlikely that these solutions give rise to any significant pain

In one experiment creatinine creatine and sodium citrate were tested concurrently No solution representing the basal pain of the method was included in this run The pain respon ses to creatinine and creatine were, numerically, exceptionally low and differed significantly from the response to sodium citrate ($p < 0\ 001$ both for intensity and duration) Compared with creatinine therefore sodium citrate had a pain produc ing effect

CHAPTER 10

HISTAMINE, SEROTONIN AND ACETYLCHOLINE

Each of these substances was added to standard salt solution (see page 32) in concentrations of 10^{6} to 10^{3} g/ml. They were tested in the usual way with 40 injections of each solution in a total of 20 subjects. Not all of them were tested concurrently, however, there being several runs of experiments. The results are shown in table 7.

Table 7 Average pain responses to solutions of histamine, acetylcholine and serotonin in different concentrations. Each solution tested on 20 subjects and injected 40 times. Those tested concurrently have identical serial numbers.

SOLUTION CONCENTRATION IN G/ML	SERIES NO	PAIN INTENSITY UNITS	PAIN DURATION SECONDS
Histamine			
10^{6}	9	1.43	12
10^{5}	9	1.50	11
10^{4}	8	2.29	15
10^{3}	8	2.49	18
Serotonin			
10^{5}	9	1.77	14
10^{4}	9	2.06	14
10^{3}	9	2.12	13
Acetylcholine			
10^{4}	8	1.61	11
10^{3}	8	2.01	10

These substances, even in relatively high concentrations, had little or no pain producing effect A noteworthy finding is that with the above solutions, histamine included, itching rarely arose, no more often in fact than with any other test solutions Histamine was tested in two different runs, the concentrations of 10^{5} and 10^{6} g/ml concurrently with isotonic sodium chlor ide solution In these concentrations it produced no pain re sponses diverging from the basal pain The concentrations of 10^{3} and 10^{4} g/ml were tested concurrently with isotonic sodium acetate solution, whose pain producing effect is just as low as the basal pain On comparison with that solution, hista mine in each of these two concentrations had a significant pain producing effect ($0\,001 < p < 0\,01$), though the average pain was only slight to moderate In one subject histamine was also tested in a concentration of 10^{2} g/ml The pain responses to two injections were 1 0 and 3 0 units respectively, i e they were exceedingly moderate notwithstanding the fact that the concentration was high enough to give rise to general symp toms

Serotonin was tested in concentrations of 10^{3}, 10^{4} and 10^{5} g/ml concurrently with isotonic sodium bicarbonate solution, whose pain producing effect is no higher than the basal pain In the concentration of 10^{5} g/ml, serotonin did not differ in pain producing effect from the comparative solution At a con centration of 10^{4} g/ml the difference in pain intensity was almost significant ($0\,01 < p < 0\,05$), and in a concentration of 10^{3} g/ml the pain producing effect was significant ($0\,001 < p < 0\,01$), though the average pain was slight and of very short duration

Acetylcholine was tested in concentrations of 10^{5} and 10^{4} g/ml concurrently with isotonic sodium acetate solution No difference in pain intensity between the latter solution and acetylcholine in the concentration of 10^{4} g/ml was found At

10^{-3} g/ml the difference was almost significant ($0.01 < p < 0.05$); furthermore, the average pain induced was slight and of short duration.

HYDROGEN ION CONCENTRATION

Since it had been observed quite early in this investigation that solutions with elevated hydrogen ion concentrations had marked pain producing effects, solutions with varying hydrogen ion concentrations were tested more extensively than other factors

Alkaline buffer solutions were subjected to one run and acid buffer solutions to two runs of experiments

Alkaline Buffer Solutions

The pain producing effects of these solutions are presented in table 8 and figure 13, which show that the effects were quite moderate and that they increased somewhat with rising hydroxyl ion concentrations

Table 8 Average pain responses to alkaline ammonia buffer solutions tested on 21 subjects Each solution injected 42 times All solutions tested concurrently

pH	PAIN INTENSITY UNITS	PAIN DURATION SECONDS
7 6	1 81	21
8 1	1 98	21
8 6	2 43	32
9 1	2 61	41
9 6	2.57	46
10 1	2 83	42
10 6	2 74	46

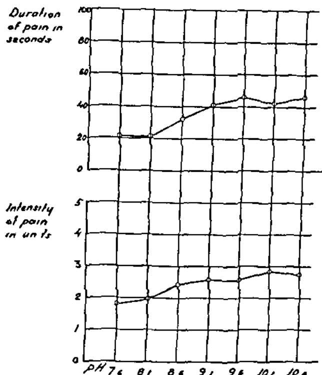


Fig 13 Diagram showing relation of pain — measured as intensity and duration — to the hydroxyl ion concentration Each point on the curves represents the mean of 42 injections in 21 subjects The buffer solutions were ammonia buffers

In evaluating the pain responses to alkaline buffer solutions, it must be borne in mind that the ammonium ion present in those solutions has, in itself, a certain pain producing effect Isotonic ammonium chloride solution, tested on 20 subjects with 40 injections, produced an average pain response of 2 05 intensity units and 24 seconds duration Though the above solution was not tested concurrently with the alkaline ammonium buffers, 18 of the subjects took part in both experiments If we compare in these 18 subjects, the pain producing effects of isotonic ammonium chloride on the one hand, and those of alkaline buffer solutions on the other, the difference is not significant until the pH reaches a level considerably higher than that normal for the blood See table 9

Acid Buffer Solutions

The pain producing effects of these solutions are shown in tables 10 and 11 together with figures 14 and 15

Table 9 Difference in pain intensity between isotonic ammonium chloride on the one hand and alkaline ammonia buffer solutions on the other

pH OF BUFFER SOLUTIONS	DIFFERENCE IN PAIN INTENSITY ON COMPARISON WITH NH_4Cl	*P* VALUE OF DIFFERENCE
7.6	None	—
8.1	None	—
8.6	Almost significant	$0.01 < p < 0.05$
9.1	Significant	$0.001 < p < 0.01$
9.6	Almost significant	$0.01 < p < 0.05$
10.1	Highly significant	$p < 0.001$
10.6	Highly significant	$p < 0.001$

Table 10 Average pain responses to acid buffer solutions tested on 16 subjects Each solution injected 32 times All solutions tested concurrently

pH	PAIN INTENSITY UNITS	PAIN DURATION SECONDS
7.2 Phosphate buffer	1.75	19
5.1 Acetate buffer	3.11	26
3.2 Acetate buffer	4.05	37
1.1 KCl HCl buffer	3.84	58

Table 11 Average pain responses to acid acetate buffer solutions tested on 20 subjects Each solution injected 40 times All solutions tested concurrently

pH	PAIN INTENSITY UNITS	PAIN DURATION SECONDS
6 2	1 81	10
5 6	2 51	14
5 1	3 34	12
4 6	3 54	12
4 1	3 86	14
3 6	3 90	17
3 2	4 18	19

It will be seen that they were pronounced, especially in respect to intensity, and rose with increasing hydrogen ion concentra tions On the whole, the effects produced in the two runs were equivalent

Regression analysis of the relationship of pain responses to the logarithmic values for hydrogen ion concentrations dis closes a significant correlation both for intensity and for dura tion in each of the two runs of experiments The following b values were obtained for the regression lines for the corre lation between pain and hydrogen ion concentration in the runs at pH 1 1 to 7 2

Pain intensity $\quad b = 0\ 74 \mp 0\ 06 \quad (p < 0\ 001)$
Pain duration $\quad b = 13\ 5 \mp 2\ 4 \quad (p < 0\ 001)$

For the runs with acetate buffer solutions alone, *the follow ing values were obtained*

Pain intensity $\quad b = 0\ 37 \mp 0\ 03 \quad (p < 0\ 001)$
Pain duration $\quad b = 1\ 3 \mp 0\ 4 \quad (0\ 001 < p < 0\ 01)$

These buffer solutions contained acetate ions as well as hydrogen ions *Acetate ions were tested separately in isotonic*

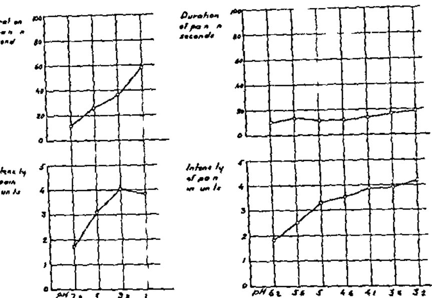


Fig 14 Diagram showing relation af pain — measured as intensity

Fig 15 Diagram showing relation of pain — measured as intensity and duration — to the hydrogen ion concentration Each point on the curves represents the mean of 40 injections in 20 subjects The buffer solutions were acetate buffers

solution in the form of sodium acetate Since this solution produced no pain that deviated from the basal pain of the method (see Chapter 9 page 46), it seems evident that the hydrogen ions constituted the pain producing factor

In the run of experiments with acid acetate buffer solutions (table 11) I tested concurrently with those solutions, an iso tonic solution containing sodium acetate with the same concen tration of acetate ions as that in the buffers (100 mN) Com parison of this solution with the buffer solution of pH 6 2 revealed a highly significant difference in pain intensity re

sponse ($p < 0.001$), hence it can be stated with assurance that a hydrogen ion concentration equivalent to pH 6 2 is pain producing

The pain then mounted with the hydrogen ion concentration of the injected buffer solution and, at pH 3 2, reached the highest intensity values recorded

The pain evoked by these acid solutions, though severe, was of very short duration; even for the most painful solution, it had disappeared after an average of 19 seconds

Relationship of Intensity to Duration of Pain

A correlation between intensity and duration of pain was found for solutions with varying hydrogen ion concentrations, as had been the case with solutions differing in osmotic pres sure and in potassium ion concentration Calculation of the regression lines for this correlation in respect of alkaline buff er solutions and acetate buffers showed a highly significant regression in each instance ($p < 0\ 001$) In figure 16 (page 60) it will be seen that the regression was far less for the acid than for the alkaline solutions The difference in the slopes of the regression lines was highly significant ($p < 0\ 001$)

Alteration of pH on Mixture of Buffer Solutions with Serum or Fluid Resembling Extracellular Fluid

On intracutaneous injection of a solution containing e g po tassium ions, some time will elapse before those ions have been dissipated by the blood circulation and by diffusion, and the potassium concentration in the tissue has returned to normal A similar mechanism may be expected on injection of buffer solution containing a high concentration of hydrogen ions though the hydrogen ion concentration may be more swiftly

affected by the buffering properties of the body and the extra cellular fluid

In order to gain some idea of the magnitude of this buffering, I conducted several experiments Four small pieces of skin were removed from autopsy cases, then denuded of subcutaneous fat and weighed Into these specimens isotonic sodium chloride solution was injected by the jet injector, producing wheals throughout the skin The specimens were then weighed again and the total water content, the fat content, and the dry weight were determined On the basis of these data it was calculated that the amount of fluid taken up was equivalent to 70—80 per cent of the amount originally present in the specimens On the assumption that some of the intracellular fluid does not mix with the injected solution, it may be roughly estimated that the latter mixes with extracellular fluid in approximately the ratio of one to one

Some of the acid buffer solutions used in the tests were subsequently mixed, in the ratio of 1 1, with serum and with a fluid resembling extracellular fluid The extracellular like fluid was prepared by mixing one part of serum with six parts of isotonic sodium chloride solution, the resulting mixture having a protein content approximating that of extracellular fluid and a pH of 6 9

The following alterations of pH after mixing were observed, each pH value being the mean from three experiments

BUFFER SOLUTION	ADDED SOLUTION PROPORTION 1 1	pH OF BUFFER SOLUTION	pH AFTER ADMIXTURE
KCl HCl buffer	Extracellular fluid	1 1	1 5
Acetate buffer	,	3.2	3 9
	"	5 1	5 5
	Serum	3.2	4 1
	,	5 1	5 9

sponse ($p < 0\ 001$), hence it can be stated with assurance that a hydrogen ion concentration equivalent to pH 6 2 is pain producing

The pain then mounted with the hydrogen ion concentration of the injected buffer solution and, at pH 3 2, reached the highest intensity values recorded

The pain evoked by these acid solutions, though severe, was of very short duration, even for the most painful solution, it had disappeared after an average of 19 seconds

Relationship of Intensity to Duration of Pain

A correlation between intensity and duration of pain was found for solutions with varying hydrogen ion concentrations, as had been the case with solutions differing in osmotic pres sure and in potassium ion concentration Calculation of the regression lines for this correlation in respect of alkaline buff er solutions and acetate buffers showed a highly significant regression in each instance ($p < 0\ 001$) In figure 16 (page 60) it will be seen that the regression was far less for the acid than for the alkaline solutions The difference in the slopes of the regression lines was highly significant ($p < 0\ 001$)

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The following alterations of pH after mixing were observed each pH value being the mean from three experiments

BUFFER SOLUTION	ADDED SOLUTION PROPORTION 1 1	pH OF BUFFER SOLUTION	pH AFTER ADMIXTURE
KCl HCl buffer	Extracellular fluid	1.1	1.5
Acetate buffer		3.2	3.9
		5.1	5.5
	Serum	3.2	4.1
		5.1	5.9

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CHAPTER 12

RELATIONSHIP OF INTENSITY TO DURATION OF PAIN

With the procedure employed, the pain responses to each test solution consisted of two factors the pain intensity and the pain duration Figures 10 to 15 (pages 39, 45, 52, 55) indicate that on the whole these two factors showed parallel variations with changes in potency of the pain producing agent Regression analysis of this relationship discloses for hypertonic, hypotonic, alkaline, acid and potassium ion containing solutions, a highly significant correlation between intensity and duration of pain ($p < 0.001$ in each instance)

From figure 16, in which the regression lines for the various solutions are collected, it is evident that the correlation varies substantially for the different types of solutions The regression is greater for hypertonic than for acid solutions, the duration at a given intensity being approximately four times longer for a hypertonic than for an acid solution

It follows that intensity and duration of pain differ in precision as criteria of the pain producing effect of a test solution The lower the *b* value, the more precisely does the pain intensity reflect the magnitude of the painful stimulus applied For the types of solutions tested in this investigation the pain intensity was in all cases the more precise of the two criteria This is evident from the *t* tests of the *b* lines for the correlation between painful stimulus and intensity or duration of pain The p values were invariably lower for intensity than for duration

No far-reaching conclusions can be drawn from these experiments, in which the tested fluids did not come into contact with the circulating blood and in which the bicarbonate-carbonate buffer was not in action. In the above-mentioned mixtures the hydrogen ion concentration of the buffer solutions was invariably reduced.

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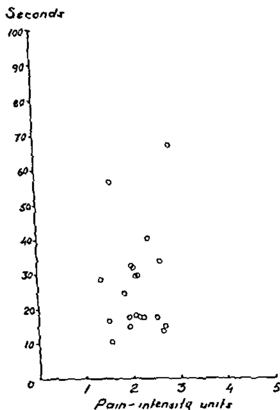


Fig 17 Diagram showing average pain intensity and duration in 20 subjects who had received identical injections of 48 *different test solutions* Each point represents the mean of 96 injections in one *subject*

On comparing the average intensity and *duration* of the pain responses in the individual subjects it is found that those two factors are not correlated but vary quite independently of each other, i e, a high as well as a low intensity may coincide with either a long or a short duration (*figure 17*) *This* behavior of the pain responses lends support to the view *that pain intensity* and pain duration are conditioned by different mechanisms

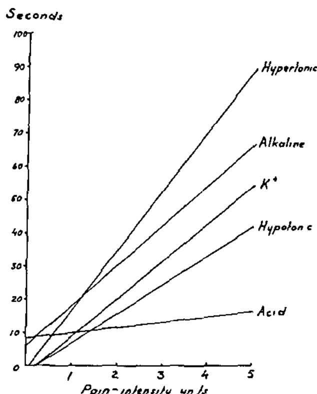


Fig 16 Regression lines for the relation of intensity to duration of pain with different types of pain producing solutions The line for hypertonic solutions is based on 180 injections of six solutions in 15 subjects (*b* value 1 83), that for hypotonic solutions, on 210 injections of six solutions in 20 subjects (*b* value 0 90), that for potassium ion containing solutions, on 320 injections of eight solutions in 20 subjects (*b* value 1 15), that for alkaline solutions, on 280 injections of seven solutions in 20 subjects (*b* value 1 22), and that for acid solutions, on 280 injections of acetate buffer solutions in 20 subjects (*b* value 0 17)

Pain intensity and pain duration seem to reflect different mechanisms The intensity may be regarded as a criterion not only of the agent's pain producing effect but of the subject's individual sensitivity The duration presumably is more dependent on local peripheral conditions such as blood circulation, buffering capacity of the tissue, etc In the discussion the main emphasis will be on the behavior of the pain intensity.

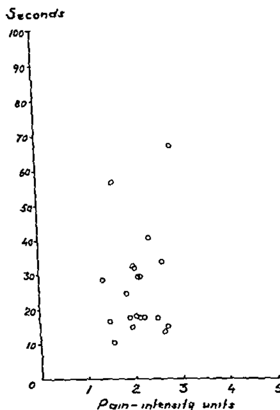


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CHAPTER 13 DISCUSSION

Pain can be experimentally elicited by mechanical, thermal, electrical or chemical stimuli To these must be added all of the various means whereby pain may arise in disease.

No generally accepted explanation exists as to how such varying stimuli can give rise to identical subjective sensations An explanation would emerge if these different pain mechanisms could be traced back to a single common mechanism In this connection I have assumed, as a working hypothesis, that all the various mechanisms might be traced back to a chemical change occurring in the vicinity of the nerve terminals This idea is based largely upon the investigations of *Lewis et al* [53, 54] concerning muscle pain Lewis assumed that such pain arises from the formation of some chemical substance (pain factor) in working anoxic muscle

If such a substance exists, it should (1) be demonstrable in most tissues, particularly the skin, in normal or painful conditions, and (2) be pain producing in concentrations equivalent to those occurring in the organism

In the following the present experimental results will be considered on the basis of this hypothesis

Osmotic Pressure

Both depression and elevation of the osmotic pressure relative to that of the body give rise to pain, provided the alteration is

of sufficient magnitude This finding verifies previous invest igatory results It has now been possible, however, to establish the range of pain inducing pressures more precisely than before

The osmotic pressure in the organism is normally fairly constant In extreme disturbances of the fluid balance, depres sion of the osmotic pressure to 203 milliosmol/l and elevation to 375 milliosmol/l have been recorded[80] A glance at tables 2 and 3 (pages 38 and 40) shows that such pressure changes do not give rise to appreciable pain

Somewhat greater osmotic pressure *shifts have been found* in cases of local pathologic conditions, particularly inflamma tion and necrosis *Ritter* [1 72] determined the reduction of the freezing point in pus from a series of furuncles He found that the osmotic pressure was usually 1 1—1 4 *times the normal* (310—434 milliosmol/l) In a few cases it amounted to 2 5 times the normal (775 milliosmol/l) Similar experimental elevation of the osmotic pressure, however, produced no appreciable pain

With a moderate reduction of the osmotic pressure, injec tion of hypotonic solutions causes no pain, not until the pres sure falls to 121 milliosmol/l or less does pain result, and thereafter it rises steeply One plausible interpretation of this finding is that the cells in the skin like the red blood corpusc les are able in some degree to resist falls in osmotic pressure Red blood cells are hemolyzed normally at an osmotic pressure of 146—166 milliosmol/l An increase of pain does in fact coincide with reduction of the osmotic pressure below those values hence it might be surmised that the pain arises when the cells are disrupted and their *contents* come into contact with the nerve terminals This hypothesis would indicate that the cell content itself was pain producing

Inorganic Ions

Of various ions tested (H_4N^+, Na^+, K^+, Ca^{++}, Mg^{++}, Cl^-, HCO_3^-, HPO_4^{--}, and SO_4^{--}) only potassium induced appreciable pain Table 12 shows the inorganic ion concentrations found in serum

Table 12 Serum content of inorganic ions Figures in parenthesis are bibliographic reference numbers

ION	TEST CONCENTRATION mN	NORMAL SERUM CONCENTRATION mN	PATHOLOGIC VARIATION mN
H_4N^+	150	0 06-0 12 (56)	—
Na^+	150	137-148 (38)	113-174 (38)
K^+	5—99	4-6 (38)	2-15 (38)
Ca^{++}	113	4 5-5 5 (38)	2-15 (38)
Mg^{++}	132	1 4-2 4 (56)	—
Cl^-	150	96-107 (38)	28-169 (38)
HCO_3^-	175	22-30 (38)	1-60 (38)
HPO_4^{--}	65	1 6-2 6 (56)	—
SO_4^{--}	66	0 7-1 2 (56)	—

Though the concentrations tested were usually far higher than those in serum—in some instances, 1,000 times higher—they still caused no appreciable pain

Quite different, however, was the behavior of potassium, whose pain inducing properties have long been recognized[32] In this investigation the pain elicited at a potassium ion concentration of 32 mN was significant even though of short duration and classed as less than slight Indeed, even at the highest concentration tested (99 mN), the average pain intensity was less than moderate

A potassium concentration of between 19 and 21 mN has

been found in normal skin[69, 84] In psoriatic skin the potassium content is elevated to 37 mN[69] A K^+concentration of 7—12 mN has been demonstrated in inflammatory exudate[60], and of 6—52 mN in pus[42], the content rising with the intensity of the inflammation These values hardly support the view that the potassium ion concentration is that factor which elicits the pain in inflammation

Organic Metabolites

Of the various metabolites tested (creatinine, creatine, sodium citrate, sodium lactate, sodium acetate, sodium succinate and sodium pyruvate), sodium citrate alone gave rise to pain Scrutiny of table 13 reveals that the tested concentrations of these substances exceeded the normal serum concentrations by approximately 100—1,000 times

Table 13 Serum content of some organic metabolites Figures in paren are bibliographic reference numbers

UBSTANCE	TEST CONCENTRATION mM	NORMAL SERUM CONCENTRATION mM	PATHOLOGIC VARIATION mM
Creatinine	272	0 05—0 10 (56)	—
Creatine	99	Traces in muscle, 23-38 (36)	—
Sodium citrate	108	0 10-0 14 (38, 56)	0 05-0 52 (38)
Sodium lactate	218	0 9-1 7 (56, 79)	4 5-26 3 (10, 5
Sodium acetate	151	Traces	—
Sodium succinate	101	Traces	—
Sodium pyruvate	153	0 03-0 15 (56, 79)	0 03-0 85 (10,

Thus I was unable to verify *Fleckenstein's*[26] observation that pyruvic acid, succinic acid and citric acid (neutralized isotonic solution) in concentrations of one half to one twentieth of that

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The concentrations of these substances in different tissues are set forth in table 14, from which it will be observed that the levels *required for inducing pain are* 100—1,000 *times* higher than those concentrations *found in* the tissue This fact militates against the view that any one of these substances acts as a *chemical mediator in* the elicitation *of pain* Yet it is conceivable that, even if a tissue contains a low total concentration of the substance, considerably higher and perhaps painful concentrations may arise locally near a surface or in a membrane To some extent, therefore, the question must be left open

Hydrogen Ion Concentration

Reduction of the hydrogen ion concentration does not induce significant pain until the concentration has fallen to a level well below that normal for the body, and the pain subsequently rises in moderate degree with a rising hydroxyl ion *concentration* Since the organism is not known to be subject to any major deviations of pH in this direction, such a *deviation* cannot be regarded as a cause of pain

Flevation of the hydrogen ion concentration gave rise to significant pain at pH 6 2 The pain thereafter *increased in* proportion to the hydrogen ion concentration, reaching at pH 3 2 the highest average intensity values *recorded* The same behavior was observed in both runs of experiments with *acid* buffer solutions A buffer solution of pH 1 1 was included in one of these runs Although an accentuation of the *pain response* might have been expected here, the response was, *in* fact largely the same for pH 3 2 as for pH 1 1 There are several possible ways of accounting for this I am *inclined to* believe that the painful stimulus to the receptors is already maximal at pH 3 2, so that a further rise in the *hydrogen ion* concentration does not increase the pain *intensity* but simply

tested here, give rise to appreciable pain. From my results it appears unlikely that these metabolites play a major role in the elicitation of pain.

Histamine, Serotonin, and Acetylcholine

In recent decades these substances have attracted considerable interest because of their high biologic activity. All three, moreover, have been ascribed pain-producing properties; indeed, in some investigations, even very low concentrations have been found to induce pain (e.g. histamine in a concentration of 10^{-18} g/g). My experiments confirm that these substances are pain-producing in concentrations of 10^{4} to 10^{3} g/ml but not below. The pain induced at those concentrations is, however, slight and of short duration.

Table 14 Histamine, serotonin and acetylcholine content in various tissues.

TISSUE	SUBSTANCE	CONCENTRATION G/ML	BIBLIOGRAPHIC REFERENCE NO
Skin, rat	Histamine	2.6×10^{5}	31
Skin, human	,,	10^{6}-10^{5}	94
Small intestine, human	,,	4×10^{5}-10^{4}	94
Diverse tissues, animal	Serotonin	10^{7}-10^{4}	18, 19, 52
Carcinoid tumor, human	,,	6×10^{4}-2.5×10^{3}	52
Snake venom	,,	3×10^{3}	52
Nerves, animal	Acetylcholine	10^{8}-10^{6}	24, 27
Brain, animal	,,	10^{6}	24
Cerebrospinal fluid, human	,,	10^{6}	80, 91
Non-nervous tissues	,,	Very low or indeterminable	24, 57

Muscle postinflammatory necrosis, guinea pig	5 0	28
Kidney, normal, guinea pig	6 7-6 9	40
Kidney, anoxic, guinea pig	6 0	40
Muscle, normal, guinea pig	7 0	61
Muscle, exhausted, guinea pig	5 8	61
Inflammatory exudate, dog	6 5-7 4	59, 60
Fracture hematoma, dog	4 7	65
Abscess, subcutaneous, dog	5 2-6 1	68
Intracellular pH		
Kidney, human	5 0 7 1	17
Gastric mucosa, human	6 5-7 0	17
Liver human	5 0-7 1	17
Pancreas, human	5 0-7 2	17
Muscle, normal, human and animal	7 1	17
Muscle, exhausted, animal	6 3	17
Muscle, exhausted and rigid animal	5 0-7 0	17
Muscle normal dog	6 6-7 1	92
Muscle normal cat	6 9	93

Scrutiny of the tabulated figures indicates that the pH may vary substantially according to e g the type and condition of the tissue etc Interstitially and in healthy tissue the pH is usually in the vicinity of the normal blood value The intracellular pH is in general lower, with values down to 5 0 It may also fall

values around 5 0 These figures indicate that elevation of the hydrogen ion concentration to a level which, experimentally, is pain producing (pH 6 2) occurs both intracellularly under normal conditions and in anoxic, damaged, or inflamed tissue

prolongs the duration This seems to be evidenced by the fact that at pH 3 2 the pain duration was 37 seconds, while at pH 1 1 it was 58 seconds

In evaluating pain responses to solutions with elevated hydrogen ion concentrations it is, as with other solutions, worthwhile to compare the hydrogen ion concentrations demonstrable in the organism In this connection see table 15

Table 15

TISSUE OR ORGAN	pH	BIBLIOGRAPHIC REFERENCE NO
Arterial blood, normal, human	7 35-7 45	56
Venous blood, chronic nephritis, human	7 05	44
Subcutaneous tissue, normal, human	7 3	70
Synovia, normal, human	7 35	78
Cancellous bone, human	7 6	78
Muscle, normal, human	7 45	78
Connective tissue, normal, human	7 45	78
Connective tissue, muscle, inflamed human	5 7-7 2	78
Tumor tissue, painful, human	5 5-6 1	70
Tumor tissue, painless, human	7 6-8 8	70
Pus from abscess, human	5 5-6 0	39
Liver, normal, dog	7 10	6
Liver, circulation arrested, dog	6 30	6
Muscle, resting, guinea pig	7 3-7 4	28
Muscle, working, guinea pig	6 5	28
Muscle, chemically inflamed, guinea pig	6 0 6 5	28

Muscle, postinflammatory necrosis, guinea pig	5 0	28
Kidney, normal, guinea pig	6 7-6 9	40
Kidney, anoxic, guinea pig	6 0	40
Muscle, normal, guinea pig	7 0	61
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Pus from abscess, human	5 5 6 0	39
Liver, normal, dog	7 10	6
Liver, circulation arrested, dog	6 30	6
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The hydrogen *ion concentration in these conditions may some* times be as much as 16 times higher (pH 5 0) A buffer solution of pH 5 1 evokes moderate to severe pain (intensity 3 34 units) Severe and even very severe pain (4 0 and 5 0 units) may of course attend inflammation and injuries, but pH values equalling those which experimentally give rise to such high pain intensities (pH 4 1 — 3 6 — 3 2) have not been demonstrable in fluids or tissues either under normal or pathologic conditions

Can an elevated hydrogen ion concentration, in these circumstances, be regarded as a cause of pain of all intensities? In the case of other tested substances such as the potassium ion or histamine, it is known that — disregarding immediate dilution by extracellular fluid — the concentration injected will persist in the tissue for a time The concentration there after decreases gradually via diffusion, in which connection the blood circulation is an important factor in removal of the ions present in excessive concentrations

In the case of hydrogen ions, the situation is different By means of *various buffering mechanisms the hydrogen ion* concentration in the blood and the body is maintained at a relatively constant level In the blood, hemoglobin, serum protein, bicarbonate carbonate and monophosphate diphosphate are the principal buffer systems[81] In principle the same buffering mechanisms exist, via the blood circulation, in the tissues It may be assumed, furthermore, that in most tissues buffering takes place via exchange of intracellular potassium for hydrogen ions[25 82]

If, therefore, a buffer solution of pH 3 2 is injected into a tissue, the relevant hydrogen ion concentration can scarcely be expected to exist for more than a very short time, via the buffering mechanisms of the body, it will be swiftly lowered towards the normal level The velocity of this fall and the time

required for normalization of the tissue pH, are not known, and indeed they would be very difficult to elucidate with available apparatus True, microelectrodes have been developed for pH determination, but they are not fine enough for intracutaneous use in measuring pH changes during and after the production of a wheal

Bjorn[9] determined the pH in rabbit subcutis following subcutaneous injections of acid solutions of local anesthetics He found that 30—45 minutes elapsed before the pH was normalized At the initial determination a few minutes after the injection, the pH had fallen by an average of one unit

My own experiments with mixtures of buffer solutions and serum or extracellular like fluid, pointed in the same direction Normalization of the pH may be expected to proceed far more swiftly in the highly vascular cutis In view of these facts, therefore, the pH values of the buffer solutions were scarcely equivalent to the hydrogen ion concentrations actually being tested, namely, those in the skin immediately after the injection The concentrations actually tested were invariably lower — probably far lower and rapidly falling Some support for this assumption lies in the fact that the pain evoked by acid solutions — in contrast to that produced by other solutions — is dissipated with the greatest rapidity, even at the highest intensities it has vanished after an average of 19 seconds

When, on the other hand, a pH of 6 2 is found in inflammatory exudate, one knows that the value remains constant over a long period In a buffer solution of pH 6 2, however, the hydrogen ion concentration will probably be lowered towards normal very swiftly This difference must be borne in mind when comparing pH values *determined* in tissues with those *tested by means of buffer* solutions

In my view the fact that severe and very severe pain is not, on the average, produced by buffer solutions until their hydro-

gen ion concentrations exceed those demonstrable in the body, does not necessarily conflict with the possibility that an elevated hydrogen ion concentration in the organism may be a cause even of severe and very severe pain.

In view of the above considerations the hydrogen ion concentration may be described as the only one of the factors investigated which satisfies the aforementioned desiderata for a chemical mediator of pain; namely, that (1) it must be demonstrable in most tissues under normal or painful conditions, and (2) it must be pain-producing in the concentrations which may occur in the organism. On the basis of this statement it may be of interest to discuss in greater detail the behavior, both actual and hypothetical, of the hydrogen ion concentration in various painful conditions, cutaneous and otherwise.

Pain can be induced experimentally not only by electrical means but by chemical, thermal and mechanical stimuli. What are the conceivable ways in which these might be traced back to an altered hydrogen ion concentration? No determinations of the cutaneous pH associated with these forms of stimuli have been reported. Hypothesizing first a low intracellular pH (6 to 5), it would seem reasonable to assume that chemical, thermal, and mechanical stimuli could disrupt the cells to such an extent that their acid contents would come into contact with the nerve terminals. Since, moreover, the cellular content is rich in protein and hence has a high buffering capacity, its pH value may be assumed to persist for a relatively long period. This view is supported in some measure by the fact that the pain evoked by injection of distilled water — which must be assumed to have caused disruption of the cells and release of their contents — was among the most severe recorded. The pain was equal in intensity to that produced by the most acid of the buffer solutions, and its duration was twice as long.

There are numerous painful conditions and diseases in

which an elevated hydrogen ion concentration has been observed A type example is the anoxic pain associated with e g angina pectoris and intermittent claudication, conditions in which elevation of the hydrogen ion concentration is known to occur via a largely glycolytic intracellular metabolism[20] A low pH has been demonstrated in anoxic skeletal muscle[28], anoxic heart muscle[20], anoxic kidney tissue[40], and anoxic liver tissue[6] A reduced pH also results from the formation of acid metabolites during heavy muscle exercise with a normal blood circulation[28 61] — An increased hydrogen ion concentration with pH 4 7 has been demonstrated in fracture hematomas[65]

Moore[62] was able to elicit in animals, by intra arterial injection of acid buffer solutions, an increased reflex activity as well as vocal manifestations as indications of pain

The pain associated with gastric or duodenal ulcer has been studied by several authors, who conclude that such *pain* is attributable to acidity of the gastric contents and subsides with alkalization of the latter[12 63 64]

A low pH has been found in tissue from painful tumors, in contrast to painless tumors where the pH has been normal[70]

An elevated hydrogen ion concentration has been recorded in inflamed tissue inflammatory exudate and pus[28 59 60 68 78]

Gaza[30] found that injection of alkaline solutions into painful abscesses and furuncles wherein the pH was low, eliminated the pain He also demonstrated that tuberculous abscesses (which usually are painless) had a *normal* hydrogen ion concentration and that injection of acid buffer solutions into them gave rise to pain

Although the question has not, of course, been definitely elucidated all these investigations nevertheless suggest *that the* hydrogen ion concentration *may be the* chemical *mediator* serving to trigger the pain stimulus in the nerve terminals of different tissues

Concerning the two aims of this investigation mentioned in the introduction, I would conclude that

(1) The present method of inducing and measuring experimental skin pain permits comparison of the pain-producing effects of eight concurrently tested aqueous solutions. Following statistical analysis of the data obtained, it is possible to determine the significance of observed pain and also of observed differences in the painful effects of the various solutions.

(2) This uniform method was employed to test a number of those substances which occur in the organism and are thought to be associated with pain. Various substances did in fact evoke pain, but only the hydrogen ion had pain-producing properties of such magnitude that it might reasonably be regarded as a chemical mediator of skin pain.

CHAPTER 14 | SUMMARY

Review of the Literature

The relatively few previous experimental investigations on chemically induced skin pain are outlined, as are various chemical methods of inducing skin pain Procedures used for estimation of the intensity of experimental pain are surveyed

Experimental Method

The author's experimental method is described with reference to injections of various test solutions by a special *jet injector* The injections were given intra and subcutaneously in volunteers, *eight different solutions being tested concurrently* in 15—21 subjects The estimated pain intensity was graded according to an ordinal scale ranging from 0 to 5 in half units, and the pain duration was recorded in seconds

Statistical Analysis

The statistical data and methods are reported

Basal Pain Associated with the Method

Injection of a non pain producing solution (isotonic sodium chloride) occasionally gave rise to slight to moderate pain The average pain response to the above solution was called

the basal pain associated with the method and was attributed to the fact that the fluid jet, in rare cases, injures the tissue and causes "traumatic pain" Only if the average pain response to a test solution differed significantly from this basal pain was the relevant solution considered pain producing

Standard Deviation and Reproducibility of the Method

The standard deviation of the method was determined by repeated injections of the same solution, and its reproducibility by repeated injections of the same test solutions on different occasions The standard deviation is reported The reproducibility was satisfactory

Test Solutions

A description is given of the composition and properties of the test solutions employed

Osmotic Pressure

Solutions with osmotic pressures two to six times higher than that normal for the organism gave rise to pain commensurate with the elevation of pressure Solutions with reduced osmotic pressures did not evoke pain until the level fell to 0 4 times the body normal The pain then increased proportionately with the reduction of osmotic pressure, reaching with distilled water an intensity which was one of the highest recorded in the entire investigation

Inorganic Ions

The cations and anions normally occurring in the blood were investigated With the exception of potassium, they were not pain producing in isotonic concentrations The potassium ion

induced pain proportional to the rise of concentration over and above the normal level in the blood At the highest concentration tested — approximately twenty times that in plasma — *the pain was, however, of moderate intensity*

Organic Metabolites

Creatinine, creatine, and the sodium salts of citric acid, lactic acid, acetic acid, succinic acid and pyruvic acid were tested in isotonic concentrations and as neutral salts They produced little or no pain

Histamine, Serotonin and Acetylcholine

Each of these substances caused slight pain in concentrations of 10^{4} to 10^{5} g/ml, but not in weaker concentrations None of them gave rise to pruritus

Hydrogen Ion Concentration

Alkaline buffer solutions evoked no significant pain until the pH values reached 9 1 and 10 1 Acid buffer solutions produced significant pain at pH 6 2 The pain then rose in proportion to the hydrogen ion concentration, reaching at pH 3 2 the highest average intensity values recorded in the investigation The pain duration was very short for all acid solutions

Relationship of Intensity to Duration of Pain

Each pain response is made up of an intensity factor and a duration factor With changes in the magnitude of any given painful stimulus, these two factors showed a largely parallel variation Analysis of their interrelationship showed a significant correlation This varied with different types of solutions,

though for all types both factors served as criteria of the pain magnitude

Discussion

The pain inducing properties of the test solutions are discussed The pain producing concentrations of the various factors are compared with those concentrations which may occur in the organism under normal and pathologic conditions It is concluded that of the factors investigated the hydrogen ion concentration is the only one which, at the levels which may be found in the skin, gives rise to pain Moreover, there is substantial evidence to indicate that it may act as a mediator of pain in numerous and diverse painful conditions

ZUSAMMENFASSUNG

Literaturubersicht

Es wird uber die bisherigen, verhältnismassig sparlichen Ver suche mit experimentellen Hautschmerzen berichtet, ebenso uber verschiedene Verfahren zur Erzeugung chemischen Haut schmerzes Methoden fur die Schatzung des experimentellen Schmerzes werden besprochen

Versuchsmethodik

Die Versuchsmethodik des Verfassers wird geschildert Bei ihr werden verschiedene Testlosungen mit einem Duseninjektor eingespritzt Diese Injektionen werden intra und subkutan frei willigen Versuchspersonen verabfolgt, wobei acht verschiedene Testlosungen nebeneinander an 15 bis 21 Personen gepruft werden Die Starke des Schmerzes wird in fünf Schmerzstarke einheiten mit halben Intervallen abgeschätzt und die Schmerz dauer in Sekunden gemessen

Zahlenauswertung und Statistik

Es wird uber die Auswertung der Zahlen und uber die sta tistischen Verfahren berichtet

Grundschmerz der Methode

[illegible]

[illegible] Die durchschnittliche Wirkung dieser Losung wird

though for all types both factors served as criteria of the pain magnitude.

Discussion

The pain-inducing properties of the test solutions are discussed. The pain-producing concentrations of the various factors are compared with those concentrations which may occur in the organism under normal and pathologic conditions. It is concluded that of the factors investigated the hydrogen ion concentration is the only one which, at the levels which may be found in the skin, gives rise to pain. Moreover, there is substantial evidence to indicate that it may act as a mediator of pain in numerous and diverse painful conditions.

Anorganische Ionen

Die gewöhnlichen im Blut vorkommenden Kationen und Anionen wurden untersucht. Mit Ausnahme des Kaliums verursachen sie in isotonen Konzentrationen keinen Schmerz. Das Kaliumion erzeugt einen Schmerz, der zur Konzentrationserhöhung über den normalen Gehalt des Blutes hinaus proportional ist. Bei der höchsten geprüften Konzentration, die etwa das Zwanzigfache der Konzentration des Serums betrug, ist jedoch der erzeugte Schmerz von mässiger Stärke.

Anorganische Metabolite

Kreatinin, Kreatin und die Natriumsalze der Zitronensäure, Milchsäure, Essigsäure, Bernsteinsäure und Pyrotraubensäure wurden in isotonen Konzentrationen und als neutrale Salze geprüft. Sie erzeugen keinen oder nur unbedeutenden Schmerz.

Histamin, Serotonin, Acetylcholin

Diese Stoffe erzeugen alle einen schwachen Schmerz in der Konzentration 10^{4} bis 10^{3} g/ml, aber nicht in schwächeren Konzentrationen. Keiner dieser Stoffe verursacht Jucken.

Wasserstoffionen

Pufferlösungen alkalischer Reaktion erzeugen einen signifikanten Schmerz erst bei pH-Werten zwischen 9,1 und 10,1. Bei Pufferlösungen saurer Reaktion entsteht ein signifikanter Schmerz bei pH = 6,2. Der Schmerz verstärkt sich dann proportional zur Wasserstoffionenkonzentration, bis er bei pH = 3,2 die höchsten durchschnittlichen Schmerzstärkewerte erreichte, die bei der Untersuchung beobachtet worden sind. Die Dauer des Schmerzes war bei allen sauren Lösungen sehr kurz.

als Grundschmerz der Methode bezeichnet, sie ist wahrscheinlich darauf zuruckzufuhren, dass der Flussigkeitsstrahl in einzelnen Fallen das Gewebe beschadigt und einen „Gewebeschadenschmerz" verursacht. Nur wenn sich der durchschnittliche Schmerz, der durch eine Testlosung hervorgerufen wird, signifikant von jenem Grundschmerz unterscheidet, gilt die Testlosung als Schmerz-Erzeuger.

Streuung und Reproduzierbarkeit der Methode

Durch wiederholte Einspritzungen der gleichen Losung wurde die Streuung der Methode ermittelt, und durch wiederholtes Einspritzen der gleichen Testlosung bei verschiedenen Gelegenheiten wurde die Reproduzierbarkeit nachgepruft. Es wird über die Streuung berichtet. Die Reproduzierbarkeit ist gut.

Testlosungen

Es wird uber die Zusammensetzung und die Eigenschaften der angewandten Testlosungen berichtet.

Osmotischer Druck

Losungen mit einem auf das Doppelte bis Sechsfache des im Korper vorhandenen Normalwertes erhohten osmotischen Druck erzeugen einen Schmerz, der zur Erhohung des Druckes proportional ist, wahrend Losungen mit verringertem osmotischem Druck keinen Schmerz verursachen, bevor die Erniedrigung das 0,4-fache des im Korper normalen osmotischen Druckes erreicht hat. Dann verstarkt sich der Schmerz im Verhaltnis zur Erniedrigung des osmotischen Druckes, bis er beim destillierten Wasser die starksten Schmerzstufen erreicht, die bei der Untersuchung beobachtet worden sind.

RÉSUMÉ

Aperçu de littérature

Précédemment, comparativement peu de rapports avaient été faits sur des épreuves de douleur de peau expérimentale par suite d'une réaction chimique, ainsi que sur les méthodes provoquant une telle douleur. Les méthodes d'évaluation de la force de la douleur expérimentale sont passées en revue.

Méthode d'expérimentation

Il est rendu compte de la méthode d'expérimentation de l'auteur. Différentes solutions de test sont injectées à l'aide d'un injecteur à jet direct Les injections — intracutanées ou sous-cutanées — sont faites à des personnes bénévoles, huit différentes solutions de test étant essayées sur 15 à 21 sujets. La force de la douleur est évaluée d'après un barème de cinq unités, avec des demi-intervalles, et la durée, en secondes.

Interprétation des chiffres et statistique

Il est rendu compte de l'interprétation des chiffres et des méthodes statistiques.

Douleur basale de la méthode

L'injection d'une solution indolore (solution isotonique NaCl) provoque dans des cas isolés une douleur faible ou modérée.

Beziehung zwischen Stärke und Dauer des Schmerzes

Nach jeder Injektion kann die Starke und die Dauer des Schmerzes registriert werden Bei Veranderung der Starke des Schmerzreizes variieren diese beiden Faktoren nahezu parallel Die Analyse ihres gegenseitigen Verhaltnisses zeigt eine signifikante Regression Diese ist bei verschiedenen Typen von Losungen verschieden, aber bei samtlichen Losungstypen sind die Starke und die Dauer ein Mass fur die Grosse des erzeugten Schmerzes

Erorterung

Die schmerzerzeugenden Eigenschaften der einzelnen Losungen werden erortert Dabei werden die den Schmerz verursachenden Konzentrationen der verschiedenen Faktoren mit denjenigen Konzentrationen verglichen, die im Korper unter normalen und pathologischen Verhaltnissen auftreten konnen Als Ergebnis dieser Erorterungen zieht der Verfasser den Schluss dass die Wasserstoffionenkonzentration der einzige untersuchte Faktor ist, der Schmerzen bei den Konzentrationen verursacht die in der Haut entstehen konnen, und dass viele Grunde dafur sprechen, dass die Wasserstoffionenkonzentration bei einer Menge verschiedenartiger schmerzhafter Zustande ein Vermittler des Schmerzes sein konnte

RÉSUMÉ

Aperçu de litterature

Precedemment, comparativement peu de rapports avaient ete faits sur des epreuves de douleur de peau experimentale par suite d'une reaction chimique ainsi que sur les methodes provoquant une telle douleur Les methodes d'evaluation de la force de la douleur experimentale sont passees en revue

Methode d experimentation

Il e t rendu compte de la methode d'experimentation de l'au teur Differentes solutions de test sont injectees a l'aide d'un injecteur a jet direct Les injections — intracutanees ou sous cutanees — sont faites a des personnes benevoles huit diffe rentes solutions de test etant essayees sur 15 a 21 sujets La force de la douleur est evaluee d'apres un bareme de cinq unites avec des demi intervalles et la duree, en secondes

Interpretation des chiffres et statistique

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I injection d une solution indolore (solution isotonique NaCl) provoque dans des cas isoles une douleur faible ou moderee

Beziehung zwischen Stärke und Dauer des Schmerzes

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Erorterung

Die schmerzerzeugenden Eigenschaften der einzelnen Losungen werden erörtert Dabei werden die den Schmerz verursachen *den Konzentrationen der verschiedenen Faktoren mit denjeni* gen Konzentrationen verglichen, die im Korper unter normalen und pathologischen Verhaltnissen auftreten konnen Als Er gebnis dieser Erorterungen zieht der Verfasser den Schluss, dass die Wasserstoffionenkonzentration der einzige untersuchte Faktor ist, der Schmerzen bei den Konzentrationen verursacht, die in der Haut entstehen konnen, und dass viele Grunde dafur sprechen, dass die Wasserstoffionenkonzentration bei einer Menge verschiedenartiger schmerzhafter Zustande ein Vermitt ler des Schmerzes sein konnte

plus haute concentration éprouvée, environ 20 fois celle du plasma, la douleur provoquée est, cependant, d'une force modérée.

Métabolisants organiques

La *créatinine*, la créatine *et* les sels de sodium des acides citrique, lactique, acétique, succinique et pyroracémique ont été expérimentés en des concentrations isotoniques et comme sels neutres Ils ne provoquent aucune douleur, sinon une douleur insignifiante.

Histamine, sérotonine, acétylcholine

Ces solutions provoquent toutes une faible douleur en des concentrations de 10^{-4} à 10^{3} g/ml, mais non en des concentrations plus faibles Aucune de ces substances ne provoque de prurit

Ions hydrogènes

Les solutions neutralisantes à réaction alcaline provoquent une douleur *significative* seulement à une valeur pH entre 9,1 et 10,1 Les solutions neutralisantes à *réaction acide provoquent* une douleur significative à pH 6,2. La douleur augmente *en* suite en proportion de la concentration des ions hydrogènes pour atteindre à pH 3,2 des valeurs moyennes de douleur les plus élevées enregistrées à l'examen La duree de la douleur pour toutes les solutions acides est très courte.

Relation entre la force et la durée de douleur

A chaque injection sont obtenues deux réponses, dont l'une sur la force de la douleur, et l'autre, sur sa durée. A la modification de la force d'irritation douloureuse, les deux réponses

La douleur moyenne causee par une telle solution est nommee dans la methode "douleur basale", estimee provenir de ce que dans certains cas, le jet du liquide endommage les tissus, occasionnant ainsi une "douleur de tissus endommages" Ce n'est que dans le cas ou la douleur moyenne provenant d'une solution de test s'ecarte considerablement de la dite douleur basale que la solution est consideree être douloureuse

Dispersion et stabilite de la methode

Par des injections repetees d'une même solution a ete etudiee la dispersion de la methode, et par la repetition d'injections d'une même solution dans des conditions differentes sa stabilite La stabilite est bonne

Solutions de test

Les solutions a pression osmotique 2 a 6 fois plus grande que la pression normale du corps provoquent une douleur en proportion de l'augmentation de la pression Les solutions a pression osmotique basse ne provoquent pas de douleur tant que la pression n'est pas descendue jusqu'a 0,4 de la pression osmotique normale du corps La douleur augmente ensuite en proportion de la diminution de la pression osmotique pour atteindre, apres une injection d'eau distillee, un degre de douleur des plus forts enregistres a l'examen

Ions inorganiques

Une etude a ete faite des cations et anions ordinaires se trouvant dans le sang A l'exception du potassium, ils ne provoquent pas de douleur en concentrations isotoniques L'ion de potassium cause une douleur proportionnelle a l'augmentation de la concentration excedant la teneur normale du sang A la

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varient d'habitude parallelement L'analyse de leur relation réciproque montre une régression significative Celle ci est différente pour différents types de solutions, mais pour tous ces types, les deux réponses expriment la mesure de la force de douleur provoquée

Discussion

Sont discutées les propriétes douloureuses des differentes solutions de test Les concentrations douloureuses des differents facteurs y sont comparees avec les concentrations pouvant se produire dans le corps dans des conditions normales ou pathologiques Comme resultat de cette discussion, l'auteur tire la conclusion que la concentration d'ions hydrogenes est le seul facteur etudie qui provoque la douleur parmi les concentrations pouvant se produire dans la peau, et qu'il y a beaucoup de raisons parlant en faveur de ce qu'une concentration d'ions hydrogenes pourrait être un agent de douleurs dans nombre de différents etats de souffrance

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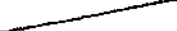
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ARYLSULPHATASE AND THE HYDROLYSIS OF SOME STEROID SULPHATES IN DEVELOPING ORGANISM AND PLACENTA

BY

MARTTI O PULKKINEN

TURKU 1961

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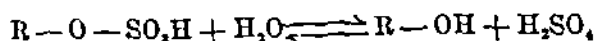
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Turku March 1961

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I
INTRODUCTION

Sulphatases are a group of esterases which split ester sulphates into sulphuric acid and the corresponding cationic group The following general formula confirms this



$$R - O - SO_3H + H_2O \rightleftharpoons R - OH + H_2SO_4$$

If we consider this reaction in another direction we are concerned with sulphate conjugation Depending on the nature of R, a different sulphatase is needed for its decomposition and the following principal groups are generally obtained

— arylsulphatase which hydrolyses aromatic and heterocyclic sulphuric acid esters,
— steroid sulphatase which is specific for the 3 β-sulphates of 5 α and Δ^5 steroids
— chondrosulphatase which hydrolyses the sulphates of chondroitin and mucoitin,
— glucosulphatase which hydrolyses the sulphuric acid esters of sugars,
— myrosulphatase which is the name given to the decomposing agent of a mustard oil glycoside The substrate is sinigrin,
— alkylsulphatase which is so far known fairly theoretical only and hydrolyses alkyl sulphates, and
— aminosulphatase or sulph amidase which participates in the hydrolysis of heparin

The sulphatase enzyme group as a whole has been studied very little in human tissues (Bianchi 1955a, Dodgson, Spencer and Wynn 1956, Huggins and Smith 1949, Neuberg and Simon 1925, Pulkkinen 1957, 1960 Rosenfeld 1925 Rutenburg Cohen and Seligman 1952, Rutenburg and Seligman 1956) Its investigation in various biological situations has been limited In the literature the main point of focus has

been pure enzymology, and the material has often been a mollusc such as *Helix pomatia, Patella vulgata* or the African land snail *Otala punctata* This is probably due in part to the speculative nature of our knowledge of the biological function of sulphatase It is known that both glucuronate and sulphate detoxication are important in the removal of toxic agents and in some other metabolic occurrences Closely associated with the latter are two of the most common sulphatases which are capable at least *in vitro* and probably also *in vivo* of hydrolysing sulphate conjugates The sulphate conjugates of steroids have a known *significance since c one third of all steroids are excreted* in the urine in compounds of this kind and for some steroids this form is probably more common than glucuronate The blood moreover obviously has higher concentrations of sulphate than of glucuronate conjugates Conjugated steroids are further said to be weaker biologically than free steroids In order to throw light on the biological function of an enzyme it must be studied in different conditions The enzyme concentrations of developing organisms in general and the localisation of the enzyme in certain organs are suitable physiological variables

An endeavour has been made to modify the methods introduced earlier in the literature to suit the requirements of the present work Both *human and animal material has been used in the investigation* itself Arylsulphatase was analysed in different tissues to establish the enzyme level The moment of the possible manifestation of sex differences was observed The possibility of splitting steroid conjugates *in the same* conditions was studied with special reference to the role of the placenta The activity of arylsulphatase was measured against p nitrophenylsulphate and of the steroid sulphates dehydroepiandosterone sulphate estrone sulphate and androsterone sulphate were investigated

II
REVIEW OF THE LITERATURE

Nomenclature and its development

The history of arylsulphatase dates back to 1911 when DERRIEN discovered in a gastropode *Morex trunculus* a factor which liberated indoxyl from indoxyl sulphate. Because of the indigo colour that originated DERRIEN gave the enzyme the name purpurase. This observation failed for some years to attract much interest. NEUBERG and KURONO found the same factor again in 1923 in a commercial powder made from *Aspergillus oryzae*. This powder was Takadiastase or Takaminase. They began to use the name sulphatase. Later on in 1932 NEUBERG and SIMON proposed the name phenolsulphatase for the enzyme to distinguish it from other sulphatases since specificity for the substrate had been observed. It was not until the 1950s that SPENCER above all suggested the name arylsulphatase a closer approximation to reality (ROBINSON, SPENCER and WILLIAMS 1951). The enzyme is not uniform. To clarify the nomenclature it may in fact be mentioned that enzyme type I of aryl sulphatase is identical with fraction C and the insoluble enzyme and that type II refers to fractions A and B the soluble fraction.

The factor hydrolysing steroid sulphate conjugates was first called alkyl sulphatase and steroid alcohol sulphatase (STITCH and HALKERSTON 1953). SAVARD, BAGNOLI and DORFMAN (1954) employed the term neutral steroid sulphatase. The present name of the enzyme steroid sulphatase was first introduced by ROY (1954) who to emphasise further the specificity of the enzyme suggested the name 3β steroid sulphatase (ROY 1956).

The following abbreviations are used in the present work

NPS	p nitrophenyl sulphate
NCS	nitrocatechol sulphate
DHAS	dehydroepiandrosterone sulphate
OS	= estrone sulphate
AS	androsterone sulphate
U_w	= enzyme unit calculated in terms of wet weight
U_N	enzyme unit calculated in terms of tissue nitrogen

Distribution

The enzyme is very widely distributed in biological sources Aryl sulphatase of type II has been found among the higher plants in the vegetable kingdom (NEY and AMMON 1959) The most recent summary of the occurence of arylsulphatase in bacteria was published by VIRTANEN (1960) For some reason the enzyme is not considered to be of great significance in the identification of bacteria

In the animal kingdom conchiferous animals and many worms contain arylsulphatase (NEY and AMMON 1959) The highest contents occurring in nature have been established in gastropods *Helix pomatia* in particular has been used as a source of the enzyme (e g JARRIGE and HENRY 1952) Although its blood liver spawn and even lungs contain aryl sulphatase (NEY and AMMON 1959) it is in the alimentary canal that the enzyme concentration is enormous This finding has in fact inspired the view that the enzyme has some digestive function in these animals

Arylsulphatase has been found in the liver of all mammalian species studied so far It was first demonstrated in the animal kingdom by NEUBERG and SIMON in 1925 They studied the liver kidney brain and muscle of man rabbit and guinea pig by using phenol ether sulphate as the substrate The mammals studied include man dog rabbit guinea pig rat mouse ox and hamster

Localisation in the different organs

Examination of the liver kidney brain and muscle of rabbit guinea pig and man have revealed that the concentration decreases in the order of enumeration (NEUBERG and SIMON 1925) By sulphatase these authors meant the ability of crude homogenate to split phenol ether sulphate

The following order of sulphatase activity was given by ROSENFELD (1925) for human organs brain kidney liver duodenum adrenal gland spleen lung small intestine muscle and pancreas He used potassium phenyl sulphate as the substrate WOHLGEMUTH (1926) demonstrated sulphatase also in human skin The following order of organs for rat using the reaction between crude homogenate and NPS which thus involved primarily the determination of fraction C was obtained liver adrenal gland kidney spleen lymph node lung

thyroid gland, prostate, testis heart, brain and, least skeletal muscle (HUGGINS and SMITH 1947) The hydrolysis of 6 benzyl 2 naphthyl sulphate by enzyme was studied in the tissues of 7 different mammalian species (RUTENBURG and SELIGMAN 1956) The tissues of hamster and rat showed the greatest sulphatase activity The tissues of man, mouse dog, guinea pig and rabbit had a much smaller sulphatase content. The liver was generally the tissue richest in enzyme, with kidney, pancreas and salivary gland next in order These workers noted a moderate content in human tissue only in the liver, kidney and pancreas The distribution of the enzyme in the organism has been studied earlier in this way

The same questions were studied with better and more modern methods by DODGSON, SPENCER and WYNN (1956) They demonstrated all the three known fractions of arylsulphatase in human liver and analysed them also in some other tissues They measured both type I and II and obtained the following order for type I liver, pancreas kidney, spleen, small intestine, lung large intestine, brain and heart The situation was roughly the same for type II, with small variations The hydrolysis of NPS has been understood here as activity of type I, and for type II the substrate used was NCS As was shown by ROY (1958), the ratio of the different sub fractions of arylsulphatase varies in the different organs of various animal species For instance there were no demonstrable quantities of fraction C in guinea pig In rat the greatest part of the sulphatase was of type B and in the ox of type A NEY and AMMON studied the different organs of *Helix pomatia* (1959) and found the enzyme in the liver lung, spawn and alimentary canal

Arylsulphatase in fluids and secreta

Arylsulphatase has been studied also in various body fluids and secreta Cow s milk and colostrum and blood contain it (VAN KOETSVELD 1955) PANTLITSCHKO and KAISER (1952) were unable to demonstrate arylsulphatase activity in human urine It can however, be considered certain that there is some arylsulphatase in human urine Thus, for instance HUGGINS and SMITH (1947) found both human serum and urine to contain some arylsulphatase The urine contains 2 different aryl sulphatases A and B which correspond to the same fractions found in human tissue (DODGSON and S[illegible]
ity of these 2 [illegible]
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serum normally show little activity against p acetylphenyl sulphate and NPS (DODGSON and SPENCER 1957a) In some conditions the urine shows a little greater activity towards these substrates although it affects chiefly the sediment depositing material Both urine and serum show considerable enzyme activity towards NCS The urine also contains a factor inhibiting pure A and B fractions This factor is thermostable and dialysable and obviously involves sulphate and phosphate ions Female urine contains an ostensibly greater quantity of sulphatase but if the debris is centrifuged off the differences disappear Urinary aryl sulphatase can be concentrated c 100 fold by acetone precipitation (AMMON and NEY 1957) The substrate used was NPS and NCS Female urine showed a higher degree of sulphatase than male urine and it increased further during menstruation The maximum age for urinary arylsulphatase was c 30 years More detailed study of serum aryl sulphatase (DODGSON and SPENCER 1954) has shown that the activity is very low Unlike fluoride and citrate heparin and oxalate exerted no effect on the determinations Determination with NPS was completed by a serum factor which hydrolyses NPS non enzymatically in an alkaline milieu The factor was thermostable and non dialysable and had no effect on the sulphates of phenol p nitrophenol and phenolphthalein Numerous sera under varying conditons were studied in the present work but the result was meagre as regards enzymatic activity According to DZIALOSZYNSKI (1957) normal urine had little activity when NCS was used as the substrate

Localisation in different tissue and cell types

The enzyme has been localised in different cell types and parts of the organs by histological methods too The results achieved so far however have been open to criticism BURTON and PEARSE (1952) for instance criticised the investigations made and pointed out that the diffusion of 6 bromo 2 naphthol liberated from the 6 bromo 2 naphthyl sulphate used as substrate was considerable and that it combined with other tissue structures which were the actual site of the enzyme SELIGMAN who used 6 bromo 2 naphthyl sulphate as substrate was the first (1950) to give information concerning the possibilities of studying the distribution of the enzyme between different cell types He noted that the enzyme was profuse in the periportal and pericentral parts of the liver It was generally present in the cytoplasm of epithelial cells but not in the

nuclei muscles or connective tissue In man the pancreas and especially the cytoplasms of the acinar cells stained heavily (RUTENBURG, COHEN and SELIGMAN 1952) The islets of Langerhans stood out from the surrounding acinar cells The epithelial cells of the ducts gave a less intensive colour than the acinar cells The activity of the adrenal gland was slight in the cortex. The most intensive colour appeared in the fascicular zone and the amount of enzyme was smallest in the transitional zone In the kidney, the enzyme was probably most profuse in the cortex The cytoplasm of the epithelial cells stained intensely here too with the exception of the glomerulus The proximal and distal parts of the tubuli were darker MALMSTROM and GLICK (1952) studied the localisation of arylsulphatase in the adrenal gland They pointed out themselves (GLICK, STECKLEIN and MALMSTROM 1955) that the results of their earlier investigation were misleading because they had neglected to take the blank values into consideration In their later investigation they established the greatest degree of activity in the fascicular and reticular zone of the adrenal gland of rat rabbit and dog but were unable to establish any activity at all in any part of cow adrenal gland They discussed in the same paper the adrenal glands of monkey and dog and the different histological zones of the adrenal gland of monkey The results regarding activity were negative for the monkey The corresponding dog organ showed relatively high activity in the fascicular zone

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Intracellular localisation

The first to devote attention to the intracellular distribution of arylsulphatase was ROY (1953a) Fractions A and B isolated from ox liver were localised in the mitochondria DODGSON, SPENCER and THOMAS (1953b) were of the opinion that the enzyme was indeed primarily

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microsomal Attention was later paid to the possibility that the different results obtained might have been due to the different substrates used One research team had used p acetylphenylsulphate, another NCS Of the different types of enzyme, type I was localised in the microsomes and type II in the mitochondria (DODGSON and SPENCER 1957b) When the homogenate was fractionated in isotonic sucrose and the localisation of the different fractions of arylsulphatase type I was compared with acid phosphatase and glucose 6 phosphatase, the percentages illustrative of activity concurred well with the corresponding values of glucose 6 phosphatase (GIANETTO and VIALA 1955) Acid phosphatase on the *other hand was largely localised in the light mitochondrial fraction* which has relatively little activity towards p acetylphenylsulphate In another investigation, in 1955, VIALA and GIANETTO studied fractions A and B and observed their mitochondrial localisation They found that it was fully analogous to that of acid phosphatase

Fractions A and B are found to some extent in microsomes in addition to mitochondria (ROY 1958), but sulphatase C occurs only in micro somes

Enzyme fractions

The complicated nature of arylsulphatase was considered in detail for *the first time in 1953 when ROY established two fractions* from an aqueous extract of an acetone powder of ox liver by fractional precipitation with acetone He called these fractions A and B, they differed from one another in their optimal pH, optimal substrate concentration, activators and inhibitors This activity was localised in *the mitochondria* Fraction C was also isolated from ox liver (ROY 1956c) The corresponding fractions have been isolated from rat liver too (DODGSON, SPENCER, and THOMAS 1955) Two different fractions debris and supernatant, were obtained by centrifuging the homogenate obtained from the acetone powder of mammalian livers (*DODGSON SPENCER and* THOMAS 1954) The former had a strong affinity for NPS and p acetyl phenyl sulphate which the latter did not split The behaviour against NCS was the reverse The results of the fractionating centrifugation were similar Two different enzymes could be distinguished one for its ready solubility after distribution of the mitochondria by acetone drying, incubation in hyper and hypotonic solutions or alternate freezing and thawing The soluble rat enzyme, again, could be distinguished into two fractions corresponding to fractions A and B

Human liver and many other tissues contain all three arylsulphatases (DODGSON, SPENCER and WYNN 1956) The A and B parts were separated by paper electrophoresis from the soluble fraction The B fraction moved more rapidly to the cathode Attempts to separate completely the soluble and insoluble part of the enzyme from one another have failed Human liver arylsulphatase B can be considerably purified by a procedure involving acetone fraction treatment with protamine sulphate and adsorption of the enzyme on insintered glass disks (DODGSON and WYNN 1958) All three fractions of ox liver have also been purified (ROY 1953b 1954a 1956c) It has not been possible yet however to obtain the enzyme in fully purified form

Arylsulphatase A of ox liver has been purified 100-fold (ROY 1953b) There is also a method for the partial purification of sulphatase B (ROY 1954a) It has been shown in paper electrophoretic studies (ROY 1954a) that sulphatase A and B differ from esterase and phosphatase Purified ox sulphatase C fraction has been observed to be very insoluble and it has been impossible to solubilise it (ROY 1956e) Fractions of human tissue have also been purified (SPENCER and WYNN 1955)

Study of the affinity of various fractions for different substrates has shown that fractions A and B possess a great affinity for NCS (DODGSON SPENCER and THOMAS 1955 ROY 1953b) Ox sulphatase A hydrolyses simpler arylsulphates in a much smaller degree than it hydrolyses NCS (ROY 1953b) Sulphatase C isolated from ox possesses a great affinity for NPS (ROY 1956b) The same applied to the preparations of sulphatase C isolated by DODGSON The debris fraction in the liver of mammals split NPS and p-acetylphenylsulphate but the supernatant did not Its reaction with NCS was the opposite The insoluble enzyme has a high affinity for NPS and acetylphenylsulphate while the soluble enzyme has a low affinity for these substrates but a higher activity towards NCS (DODGSON SPENCER and THOMAS 1955) The NPS curves of fraction A purified by means of paper electrophoresis show that this enzyme has a low affinity for this substrate and that its kinetic behaviour differs markedly from that of the insoluble enzyme The NPS activity of enzyme B was almost negligible (DODGSON SPENCER and WYNN 1956a) Human liver arylsulphatase B was active for 2 hydroxy 5 nitrophenylsulphate (NCS) but showed little activity towards potassium phenyl sulphate and its monosubstituted derivates there was appreciable activity towards disubstituted derivates (DODGSON and WYNN 1958)

Optimum determination conditions

The optimum enzymological conditions for the determination of aryl sulphatase vary with the substrate or buffer used the source of the enzyme, and the activating or inhibiting ions involved, in certain cases even the enzyme concentration used influences the reaction conditions e g fraction A (DODGSON and SPENCER 1956a) The optimum conditions for assay of arylsulphatases from various sources have been reviewed by DODGSON and SPENCER (1957a) They compiled two tables for the changing of these different variables in relation to one another The present author has concentrated here on earlier findings concerning the optimum conditions for this investigation HUGGINS and SMITH (1947) established 6 12 as the optimum pH of rat liver at 37°C with 0 5 N acetate buffer and 0 015 M as the optimum substrate concentration when p nitrophenyl sulphate was used Using an unknown substrate concentration, ABBOT and EAST (1949) established 6 6 as the optimum pH for rat liver and kidney and 50°C as a suitable incubation temperature The arylsulphatase activity of the adrenal gland of rat towards NPS was measured in acetate buffer, pH 5 8 using a substrate concentration of 0 0005 M (GLICK, STECKLEIN and MALMSTROM 1955) These earlier investigations were performed with whole homogenate For purified rat arylsulphatase C, using NPS as the substrate in 0 5 M acetate buffer, the optima are pH 7 0 and substrate concentration 0 006 M (DODGSON, SPENCER and THOMAS 1955) For fraction C isolated from human tissues the optima are 7 3 and 0 008 (DODGSON, SPENCER and WYNN 1956a) For rat arylsulphatase A and B with NPS the optima are 5 8—0 12 and 6 2—0 12, and for human arylsulphatase A 6 2 and 0 07

Inhibition and activation of the enzyme

By may of a general statement it can be said that arylsulphatase of type I is inhibited by cyanide but hardly affected by phosphate or sulphate Type II, on the other hand is unaffected by cyanide but is strongly inhibited by phosphate and sulphate (DODGSON and SPENCER 1957a) Inorganic phosphate is identified as an endogenous non competitive inhibitor of type II (MAENGWYN DAVIES and FRIEDENWALD 1954) Substantially all of the inorganic phosphate is in the undialysed preparation The activity of freshly thawed but undialysed preparations

was found to be markly less than that of similar preparations after exhaustive dialysis against running tap water Activation by some of the heavy metals may presumably be due to removal of the inorganic phosphate left in the preparation after dialysis This endogenous inhibition lends interest also to the ratio between acid phosphatase and arylsulphatase Endogenous inhibition attracted the attention of RUTENBURG and SELIGMAN (1956) also The high endogenous phosphate content of rat kidney that they obtained produced an inhibition of enzymatic activity in acetate buffer which was greater than that noted with liver

Several workers have found quite a number of different general biological enzyme inhibitors to be suitable also for arylsulphatase (DODGSON and SPENCER 1953b DZIALOSZYNSKI 1947 1950 HOMMERBERG 1921 ROBINSON et al 1952 SELIGMAN CHAUNCEY and NACHLAS 1951 TORDA 1943) However as far as the older investigations are concerned there can be no certainty about which fraction was measured in each case The inhibition and activation of different arylsulphatase types by pure enzyme preparation was studied by the research teams of DODGSON and ROY (DODGSON and POWELL 1959 DODGSON and SPENCER 1957a DODGSON SPENCER and WYNN 1956 and ROY 1954a 1955 1956b)

Solubility of arylsulphatase

Two different arylsulphatase types differ in their solubility Fractions A and B have been demonstrated in an aqueous solution obtained from an acetone powder of ox liver These fractions were water soluble (ROY 1953b 1954a) Sulphatase C on the other hand could not be solubilised with a wide pH range of buffer and hyper or hypotonic saline solutions through alternate freezing and thawing or mechanical disruption (SPENCER et al 1955) Trypsin solubilised a part of arylsulphatase but caused a decrease in activity The enzyme could be solubilised by using either a cationic or non ionic surface-active substance resulting in creased arylsulphatase activity on account of the greater enzyme dispersion (DODGSON et al 1957b) Solubility and activation effect seem to be associated with the formation of micelle together with the solvent Anionic agents solubilised the enzyme but caused inhibition of activity of arylsulphatase Fractions A and B are readily solubilised from the mammalian liver after rupture of the mitochondria of the liver cells (VIALA and GIANETTO 1955) In this insoluble condition arylsulphatase

A and B show only a part of their total activity and the granules with which they bind become irreversibly activated when a method is used that dissolves the enzyme treatment with hypotonic sucrose solution or corresponding saline solution, mechanical disruption, freezing and thawing and incubation at 37°C together with isotonic sucrose solution Although sulphatase A and B are localised mainly in the mitochondrial and microsomal fraction, they cannot be completely separated from them with water

Mechanism of action

Something is known of the details of the structure and mechanism of action of the enzyme DODGSON, SPENCER and WILLIAMS (1956) studied the changes in the affinity between the substrate and the enzyme by binding various substituents with the benzene ring They used an aryl sulphatase obtained from *Alcaligenes metalcaligenes* The inclusion of electrophilic groups such as the nitro group increases the affinity between the enzyme and the substrate by raising the positive charge of the S atom of the residual sulphate Nucleophilic substitutes have the contrary effect These authors have published a table showing how the Michaelis constant K_m and V_{max} with phenolsulphate as the basis change when different substituents are included in the phenol ring For NPS it is 10^4 K_m 4 75 There must be an intensive "electron withdrawing" effect on the sulphate group of arylsulphate before it can be hydrolysed by arylsulphatase to any considerable extent DODGSON SPENCER and WILLIAMS introduced also a hypothetical formula for the mechanism of the enzyme action

ROY studied a number of inhibitors and came to the conclusion that arylsulphatase C is an SH enzyme (1956b) The activity of the A fraction of ox liver is not directly proportional to the concentration It seems that this effect can be explained as the polymerisation of enzyme molecules, producing a complex which is more active than the non polymerised molecule (ROY 1954b) Later he wrote that this hypothesis was not correct (ROY 1960) Sulphatase A of ox liver has, like C, been classified as probably an SH enzyme (ROY 1956b), but for B the matter is not clear There is some indication as to the nature of the ionising group that brings about the formation of the enzyme-substrate complex and the cleavage in each individual case This problem has been investigated by DODGSON and WYNN (1958) There

are two possible ways in which a compound of type Ar O SO$_2$ O splits into Ar OH and HO SO$_2$. The bond may break either between Ar O or the O—S link may split In reality, it is the latter alternative that occurs (SPENCER 1958) SPENCER hydrolysed aryl hydrogen sulphates with acid alkalis and enzymes in a solution containing H$_2$ ^{18}O The liberated sulphate was isolated and its ^{18}O activity determined The sulphate part had radioactivity i e the O—S linkage had split irrespective of the hydrolysing agent used

The possible role of arylsulphatase in conjugation involves an altogether different effect on the mechanism of action If arylsulphatases act as transferases they catalyse the transfer of the sulphate rather than of the phenolic group (SPENCER 1957) The sulphated polysaccharide of the mucous gland of *Charonia lampas* (charonin sulphate) can be sulphated with a system which contains arylsulphate arylsulphatase carbohydrate acceptor and at least one unknown factor (SUZUKI, TAKAHASHI and EGAMI 1957) This transsulphation is inhibited by phosphate and fluoride in the same manner as the hydrolysis of arylsulphates is inhibited by arylsulphatase Judging by this work of SUZUKI TAKAHASHI and EGAMI it seems that arylsulphatase really does participate itself in a reaction of this kind What seems to be involved here is the transfer of sulphate from one sulphate ester to another, a movement which is directly synthesised from PAPS, adenosine-3 phosphate 5 phosphosulphate An example is the synthesis of ^{35}S charonin sulphate and glucose-6-^{35}S — SO$_4$ from charonin sulphate and NPS under the influence of an acetone-dried preparation obtained from the mucous gland of *Charonia lampas* No transfer occurred when the p nitrophenyl ^{35}S-sulphate was replaced by ^{35}S K$_2$ SO$_4$

Arylsulphatase in different physiological conditions

Some investigations have touched upon the arylsulphatase content of the organism as a function of age Urinary excretion of arylsulphatase is fairly small in children increases later reaches its maximum at the age of 30—40 and falls again with the approach of old age (AMMON and NEY 1957) The phenolsulphatase content of the aorta and the pulmonary artery decreases with age (KIRK and DYRBYE 1956) and shows fairly good correlation with arteriosclerotic changes Arylsulphatase C has been found to be much more profuse in the liver of male than of

A and B show only a part of their total activity and the granules with which they bind become irreversibly activated when a method is used that dissolves the enzyme treatment with hypotonic sucrose solution or corresponding saline solution, mechanical disruption freezing and thawing and incubation at 37°C together with isotonic sucrose solution Although sulphatase A and B are localised mainly in the mitochondrial and microsomal fraction they cannot be completely separated from them with water

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the occurrence of arylsulphatase in man during the fetal period (PULKKINEN 1957) Rat seemed to have a tendency during its fetal development to rising arylsulphatase concentrations in the liver (HARTIALA et al 1958) no differences were observed after the livers were cultured on a fungus

Origination of substrate

The mode of origination of the substrate for arylsulphatase does not actually come into the present work A possibility that must be considered however is that arylsulphatase might perhaps be present in the chain of synthesis in certain of the special cases cited. The main principles of the present view of sulphate conjugation were introduced by FRITZ LIPMANN in 1958 The first step is the formation of active sulphate and the enzymes associated with it are common in conjugates of various types

The reaction between adenosintriphosphate and sulphate produces through the agency of sulphurylase and ATP kinase an active sulphate adenosine-3 phosphate 5 phosphosulphate From here the *sulphate is* transferred through the agency of a more or less specific *enzyme*, sulphokinase to steroid phenol etc

Phenols have a common phenolsulphokinase estrone has its own and there is again a common one for 3 β hydroxysteroids Chondroitin sulphuric acid and the cerebroid sulphate of the brains thus also *obtain* their sulphate group in the same way

If the sulphatase participates in this mechanism it obviously transfers the sulphate from one ester to another and the sulphate ester is synthesised direct from adenosine-3 phosphate-5 phosphosulphate Substrates for sulphatase originating in this way can be found in the organism

B Steroid sulphatase

Hydrolysing of steroid sulphates in the organism

Steroid sulphatase is highly *specific* capable of hydrolysing only a *small part of all steroid sulphates* If the steroid has an aromatic ring as is the case with estrone sulphate steroid is liberated by ordinary arylsulphatase It has not been possible to split 3 α-steroids, e.g androsterone sulphate by means of biological materials

female rats The mouse strain studied, on the other hand, revealed no sex difference The activity of sulphatase A and B is increased in proliferating tissue (Roy 1958) *It is extraordinary that castration has no* effect on the sex difference The activity of sulphatase A and B was very high in four young rats, while the activity of both sulphatase C and *steroid sulphatase was slightly lower than normal* Reference has been made to the sex difference in urine, and it would seem that female urine contains a greater amount of arylsulphatase C (DODGSON and SPENCER 1957a) This, however, is due to the more profuse sediment If the sediment is centrifuged off carefully, female urine has as much enzyme as male urine The same applies probably also to certain observations of a higher arylsulphatase concentration in the urine during menstruation (AMMON and NEY 1957) HAYASHI et al (1955) noticed however, that castration had a lowering effect on the arylsulphatase content of the rat uterus The cyclical changes of the enzyme in the uterine wall were definite The arylsulphatase in the apical cytoplasm in the proestrus increased on entering early estrus and changed to basal cytoplasm in the actual estrus (HAYASHI et al 1957) The time of the day has also been considered to influence the sulphatase concentration In the urine, maximum values were found at noon Serum gave the same result (BOYLAND, WALLACE and WILLIAMS 1955) The effect of nutritional factors on sulphatase activity has also been discussed (DODGSON, LEWIS and SPENCER 1953)

Rat given 50 IU of estrogen showed the highest arylsulphatase values 4 days later in the endometrium and especially in the epithelium of the *uterine lumen (HAYASHI et al 1955) Only a weak positive reaction* remained 6 months later, and the "increasing stadium' in such situations was shorter for arylsulphatase than for β glucuronidase The phenol sulphatase of guinea pig *liver rose when the animal was given estrogen* (BIANCHI 1955a) Experiments have also been conducted concerning the effect of ACTH, DOCA and cortisone on the enzyme content and the stress conditions of turpentine *abscess or cold were studied at the same* time No changes were observed histologically in the distribution of phenol sulphatase activity when the results were calculated in terms of the protein nitrogen content *(GLICK and STECKLEIN 1956) A notable* fall was observed in the activity in the fascicular and reticular zone 6 days after hypophysectomy and this effect was of almost the same order after 30 days

The present author has published previously a preliminary report on

It has been emphasised that steroid sulphatase is highly specific. It hydrolyses only the 3 β-sulphates of the 5 α and Δ^5 series. Estrone sulphate e.g. is thus hydrolysed under the influence of arylsulphatase.

Several investigators have used steroid sulphatase for splitting the urinary sulphate conjugates (COHEN and BATES 1949, HENRY and THEVENET 1952, HENRY, THEVENET and JARRIGE 1952, JAYLE and BAULIEU 1954, STITCH and HALKERSTON 1953a and b and STITCH, HALKERSTON and HILLMAN 1956). In general, no attention has been paid, however, to the relatively narrow specificity of the enzyme and the value of steroid sulphatase as a substitute for acid hydrolysis is questionable. It may be helpful in some special cases if the structure of the steroid must be kept completely unchanged. Hydrolysis of the neutral 17-oxosteroids of the urine with enzyme or acid revealed in chromatography that there were differences in the distribution of the chromatographic fractions (STITCH and HALKERSTON 1956). GIBIAN and BRATFISCH (1956) stated concerning the stability of the enzyme that c. 50 per cent of the activity remained in their acetone powder after 3 months' storage.

There is a mention from the field of physiology that rat liver displays sex differences and male rats have been found to possess more profuse steroid sulphatase than female rats but a smaller sulphate conjugation ability (ROY 1958).

The information on steroid sulphatase in mammals has been limited so far to its occurrence in the liver It has been demonstrated in guinea pig also in the spleen and the adrenal gland (GIBIAN and BRATFISH 1956) It has not been established prior to the present work in an organ so important for the steroid balance during pregnancy as the placenta The present author published a preliminary communication on the subject elsewhere (PULKKINEN 1960).

Distribution, localisation and properties

Steroid sulphatase was first found in the intestinal fluid of *Helix pomatia* (HENRY and THEVENET 1952) The steroid sulphatase in mammals was demonstrated by GIBIAN and BRATFISH (1956) They used rat and cow liver as the source of the enzyme ROY, in his investigation of a later date, worked with ox and rat liver (1957) Plants, bacteria and fungi obviously do not contain steroid sulphatase (NEY and AMMON 1959) The liver of fish and birds contains this enzyme The same investigation established steroid sulphatatic activity in the adrenal gland, liver and spleen of guinea pig It has not been possible to establish it in human urine

Steroid sulphatase is localised intracellularly in the mitochondrial fraction (ROY 1957)

Steroid sulphatase has been separated electrophoretically from other sulphatases (ROY 1956a) The optima for its determination in *Patella vulgata* enzyme were as follows pH 4 5, substrate concentration 0 2 mM The results show a good fifth of the LINEWEAVER and BURK equations and gave a value of 0 04 mM DHAS for K_m This substrate concentration has also been used with an enzyme derived from mammals (GIBIAN and BRATFISH 1956 and ROY 1957), the optimum pH, however, was higher than for *Patella* enzyme *GIBIAN and BRATFISH* determined rat liver steroid sulphatase activity at pH 7 3—7 5, using 0 5 M triethanol ammonium acetate buffer ROY used pH 7 8 with ox liver in TRIS buffer

A table was published by ROY (1954b) for the inhibition of steroid sulphatase It showed phosphate, fluoride and sulphate as the strongest *common inhibitors* The same observation was confirmed by SAVARD, BAGNOLI and DORFMAN (1954) and by GIBIAN and BRATFISH (1956) This enzyme is water *soluble*

Table I Distribution of the rat material by age and sex for arylsulphatase determination.

Sex	Age or size	Number
—	fetus of under 4 g	49
—	fetus of over 4 g	15
—	newborn	46
male	1 month	20
female	1 month	16
male	adult	30
female	adult	26
		Total 202

for legal abortion at the Women's Hospital, University of Turku the Maternity Hospital of the City of Turku and Turku Nursing Home. Three cases of diabetes comprised the only endocrinological indications for interruption of pregnancy. Fifty five placentas of fetuses of different ages were collected in the same connection. Their enzyme activity was analysed on both the maternal and fetal side. A further 29 placentas obtained from normal vaginal deliveries were collected from the Women's Hospital University of Turku.

The test animal used was the Wistar rat strain (inbred in this laboratory during the last 5 years). The animal material consisted of 65 rat fetuses of different age from 24 mothers 46 newborn (under 24 hours of age) 36 month old and 56 adult *rats aged 3 ½ months*, i e 202 animals in all. The sex distribution of the main groups was as follows 16 female rats aged 1 month 20 male rats of the same age and 26 adult female and 30 adult male rats. The distribution is shown in Table I. The rats were kept throughout the experiment on a fairly standard diet consisting of mixed foodtype. This has been found to be a *suitable* fare judging chiefly by good reproductive ability and coat of hair and lack of other deficiency signs. The material was *collected* in different seasons simultaneously from all the different groups. The fetuses were weighed and weight was used as the *criterion of the stage* of development. It is difficult to determine the duration of the pregnancy of a rat the time of *fertilisation* is always somewhat uncertain.

Arylsulphatase activity was determined from the liver, kidney and intestine

III

THE PRESENT INVESTIGATION

THE PROBLEM

The purpose of the present investigation was to study the following problems

(1) Control of the p nitrophenyl sulphate method and its application in the present study

(2) The arylsulphatase content of different organs during fetal development in man and in rat and differences between the organs in this respect

(3) The arylsulphatase activity of the different organs of rat during extrauterine development Attention was also paid to the differences between the sexes and the time they appeared

(4) The hydrolysis of arylsulphate conjugates in the placenta

(5) The hydrolysis of steroid sulphate conjugates in the developing organism of man and rat during intrauterine life

(6) The ability of growing rat to hydrolyse steroid sulphates

(7) The placenta as the producer of free steroids from their sulphate conjugates with special reference to the existence of specific steroid sulphatase

(8) The ability of hydrolysing 3α steroid sulphate by various tissues

MATERIAL

A Arylsulphatase

The material was divided into two principal groups human and animal

The human series consisted of 71 fetuses whose crown heel length ranged from 2 5 to 33 cm Twenty seven different tissues and secreta of these fetuses were analysed for arylsulphatase content as far as size permitted The material was *collected from sectio minor operations*

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Arylsulphatase activity was determined from the liver, kidney and intestine.

Table II Distribution by age and sex of rats used for determination of hydrolysis of DHAS and OS

Age	DHAS			OS		
	Male	Female	Total	Male	Female	Total
fetus	—	—	23	—	—	29
under 24 hours	*12*	*5*	*17*	12	4	*16*
1 month	14	14	28	12	15	27
adult	15	15	30	15	13	28
Total	41	*34*	*98*	*39*	*32*	*103*

B Steroid sulphates

The material was divided into two parts human and animal Table II shows the size of the groups of rat material A total of 102 animals were analysed for hydrolysis of dehydroepiandrosterone and estrone sulphate to accertain the age development

The rats were the same as and kept in identical conditions with those in the part of the work concerned with arylsulphatase

Six human fetuses were analysed, they were collected in the same way as for arylsulphatase determinations

The placentas obtained from spontaneous human deliveries 29 in all were analysed from the maternal and the fetal side for both steroid sulphates

Random samples were collected from rats human placentas parts of human digestive tract obtained at operations and from fresh human fetuses for study of the hydrolysis of androsterone sulphate The samples were treated in the same way as for the arylsulphatase determinations

METHOD

Treatment of the samples

The fetuses were placed in a $+4°$ C refrigerator immediately after the operation The different organs were prepared as carefully as possible in a room of the same temperature and the pieces of tissue selected were weighed on an analytical balance (Sartorius Selecta accuracy 0 1 mg)

The rats were killed by a blow on the neck The pieces of tissue were weighed immediately as above and kept during the manipulation at +4° C

From the placentas, sample sections were cut from the maternal side at a site with no distinct calcification A section taken from directly below the membranes was called the fetal side The blood was drained from the samples by blotting paper and the samples were treated in the routine way

A Arylsulphatase

Main principles of the determination

The earliest investigators used the gravimetric method to establish enzyme activity The unhydrolysed phenol ether sulphuric acid was hydrolysed by hydrochloric acid and the liberated sulphate was determined as the sulphate of barium or benzidine by weighing (NEUBERG 1923) Measurement of the liberated phenol was also used DODGSON and SPENCER have given an extensive summary of the different methods employed (1957a) They can be divided into three principal groups according to the component of the reaction $R-O-SO_3H + H_2O \rightarrow R\ OH + H_2SO_4$ that is to be measured It is thus possible to measure

(a) R OH
(b) H_2SO_4
(c) $R-O-SO_3H$ which failed to hydrolyse

The first of these methods is the one most commonly chosen Phenol or another similar agent can generally be most easily determined spectrophotometrically and this in fact is the most common procedure The commonest substrates of arylsulphatase are NPS and acetylphenyl sulphate (for type I) and NCS i e 2 hydroxy 5 nitrocatechol sulphate (for type II) and hence it is often necessary to determine the corresponding phenols [illegible]

be employed for th [illegible]

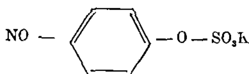
be determined in va[illegible]ous [illegible]ays

The third alternative is to measure the substrate that has failed to hydrolyse The earlier method (NEUBERG and WAGNER 1927) of hydrolysis and then determining the liberated sulphate gravimetrically involves prolonging the incubation time because there has to be a rela

tively great difference between the enzymatically hydrolysed and unhydrolysed substrate (Roy 1956a)

The method used here was based on the determination of the liberated phenol

Arylsulphatase was determined by HUGGINS SMITH's method (1947) using NPS as substrate The method was modified for the purposes of the present work



Potassium salt of p nitrophenylsulphate

Reagents

(1) Acetate buffer 68 08 g of CH_3 COONa + 3 H_2O (Merck, *p A*) was weighed ad 1 l of distilled water and allowed to dissolve over night The pH was adjusted to the desired level with 0 5 N acetic acid Four solutions were prepared pH 7 3, 7 1, 7 0 and 5 8 The pH was controlled by glass electrodes in a Beckman pH meter The pH of the solutions were checked occasionally

(2) Substrate, p nitrophenylsulphate (Sigma Chemical Co) The reagent was kept crystalline in an exsiccator at + 4°C and only the amount required daily was dissolved in water The reagent keeps fairly well in crystalline form, after several months the blank values may rise in which case a red colour is seen in the p nitrophenylsulphate When this happened the substrate was discarded For rat tissues 1 285 mg of NPS was dissolved in 1 ml of distilled water For human tissues the corresponding amount was 6 425 mg/ml

(3) Sodium hydroxide (Merck *p A*) Sodium hydroxide of two different potencies was used 0 5 N and 0 63 N

(4) 10 per cent zinc sulphate (Merck *p A*), $ZnSO_4$ + 3 H_2O

Homogenates and the reaction conditions

The weighed pieces of tissue were homogenised by POTTER ELVEHJEM's (1936) glass homogeniser in a refrigated room at + 4°C until the homogenate looked even The most important variables in the reaction conditions are shown in Table III

Table III The reaction milieu in the determination of arylsulphatase of some tissues

	Tissue	Homogenate %	Incubation time hours	pH	Substrate concentration	Normality of NaOH
Rat	liver	1	3	7 0	0 001	0.5
	kidney	0.5	,,	5 8	,,	0 63
	intestine	1	,,	7 0	,,	0.5
Man	liver	0.2	1	7.3	0 005	0 5
	kidney	1	,,	7.1	,,	,,
	intestines	,,	,,	7 3	,,	,,
	pancreas	,,	,,	,,	,,	,,
	placenta	,,	,,	,,	,,	,,
	brains	3	3	,,	,,	,,
	lungs	,,	,,	7 1	,,	,,

The wet weight percentage of the homogenates was 1, except for rat kidney for which it was 0 5, for human fetus liver 0.2 and for brains and lungs 3 The rat kidney percentage was changed because, due to the small size of the fetal kidneys, it was impossible to obtain a 1 per cent homogenate from the fetus material, hence 0 5 per cent was used throughout the work For human fetus the homogenates were changed to allow better determination of activity Three-hour incubation was considered more suitable for rat tissues For human fetuses 1 hour was sufficient for liver, kidney, intestines, pancreas and placenta, for other tissues the incubation time was 3 hours The substrate concentration used was 0 001 for rat tissues and 0 005 M for human tissues, both were below the optimum. Because of the low pH of the buffer it was necessary to use a stronger base for precipitation of rat kidney in order to achieve full intensity of colour

Determination of activity

Into 1 5 ml of acetate buffer was pipetted 0 5 ml of homogenate at the optimum pH of each sample (Table III) Ordinary test tubes were used The tubes were pre incubated for 3 min in a water bath at + 37°C The reaction was then started by adding 0 5 ml of NPS solution into the tubes to a final molarity of 0 001 in the rat analyses and of 0 005 in the analyses of human fetuses and placentas After the reaction time set for

each sample (1 hour or 3 hours), 0 8 ml of sodium hydroxide was added to the tubes to stop the reaction Into one tube at a time was added 0 5 ml of 10 per cent zinc sulphate and the tubes were shaken carefully Immediately after this the tubes were filtered by suction through Whatman No 40 filter paper (Ø 7 cm) The p nitrophenol colour that originated was measured by Beckman DU spectrophotometer at wave length 402 mμ in normal cuvettes against distilled water All determinations were made in duplicate The procedure was the same in the control determinations except that the homogenate was added to the test tube immediately before pipetting the base This was done with the various tissue types at each determination

Determination of tissue nitrogen

The tissue nitrogen of the homogenates was determined by the micro-Kjeldahl method (see e g HAWK OSER and SUMMERSON 1954) using K_2SO_4 $CaSO_4$ during the evaporation of the water and adding 30 per cent hydrogen peroxide The ammonia was distilled in boric acid and titrated with sulphuric acid to the colour change point of bromcresol green methylred mixture as indicator One ml of the 0 1 N sulphuric acid corresponded to 0 14 mg of nitrogen The method was accurate to 0 1—1 mg of nitrogen

The enzyme unit and its calculation

The unit of arylsulphatase was calculated from the following formula

$$\text{Number of units } U_w = \frac{\mu g \text{ of liberated p nitrophenol}}{100 \text{ mg w/w hours}} \text{ or here}$$

$$\frac{\mu g \text{ of liberated p nitrophenol} \quad 20}{\% \quad \text{hour}}$$

The results were also calculated per tissue nitrogen

$$\text{Number of units } U_N = \frac{\mu g \text{ of liberated p nitrophenol}}{\text{mg nitrogen hour}} \text{ or here}$$

$$\frac{\mu g \text{ of liberated p nitrophenol} \quad 2}{\text{mg nitrogen} \quad \% \quad \text{hour}}$$

The total of μg of liberated p nitrophenol was obtained by subtracting from the Beckman reading the blank value and multiplying the result by 0 0492 This figure was obtained from the calibration curve which was linear in the zone studied

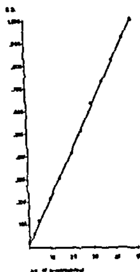


Fig 1 Calibration curves for p-nitrophenol

Re crystallised p nitrophenol (Fluka) was employed in the calibration It was performed in exactly the same way as the procedure used in enzyme determinations substituting p nitrophenol for the substrate Similar calibration curves were obtained with 1 per cent rat liver homogenate or water instead Six parallel determinations were made for both at 5 μg intervals over the range 2 5—50 μg

B Hydrolysis of steroid sulphates

Steroid sulphatase can also be determined in three different ways either liberated sulphate or steroid is determined and in the third method the residual substrate The first method has not been used, but now when there is a better method than before of determining inorganic sulphate by means of benzidine or barium chloranilate (HÄKKINEN 1959 1960) this method may be the best Liberated steroid can be determined in the routine way by the ZIMMERMAN technique (ZIMMERMAN 1936) This method was used e g by GIBIAN and BRATFISH (1956) The technique for the determination of residual substrate is also relatively simple Steroid sulphate and methylene blue combine to form a complex which can be extracted in chloroform This complex is not formed by the free steroid The method is applicable to all steroid sulphates (ROY 1956a)

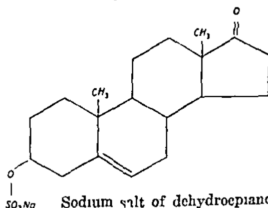
The splitting of steroid sulphates was studied here by determining the

unhydrolysed steroid sulphate as a methylene blue complex which was extracted in chloroform (Roy 1954b) and using the enzyme reaction conditions set out in the literature for rat liver (Roy 1957). Minor methodological modifications were made.

Choice of substrate

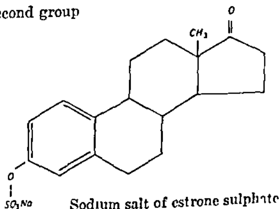
The purpose was to analyse 3 basic types of steroid sulphate:
(1) sulphate of 3 β steroid, which would be a specific substrate for steroid sulphatase
(2) Sulphate of aromatic steroid, which would be split by common aryl sulphatase
(3) Sulphate of 3 α steroid, which hardly occurs in nature and which it has not so far been possible to split biologically.

Dehydroepiandrosteron sulphate, DHAS was selected for the first group.



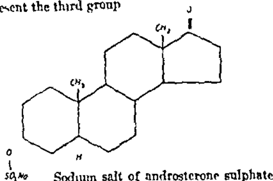
Sodium salt of dehydroepiandrosteron sulphate

DHAS has been used almost exclusively in the literature and it is hydrolysed well by specific steroid sulphatase. Estrone sulphate (OS), which was also used in the present work, has generally been used to represent the second group.



Sodium salt of estrone sulphate

Androsterone sulphate is a typical sulphate of 3 α-steroid and it has been used to represent the third group



Sodium salt of androsterone sulphate

Reagents

(1) 0 5 M TRIS, i e 2-amino-2 hydroxymethylpropane 1 3-diol-acetic acid buffer (Sigma Co) 6 05 g of TRIS was weighed to make 100 ml of the buffer Using concentrated glacial acetic acid, the solution was adjusted to pH 7 8

(2) 0 4 mM steroid sulphate The steroid sulphates used were sulphates of dehydroepiandrosterone, estrone and androsterone (Schering Ag) DHAS and AS were crystalline in ampules, but OS was dissolved in triethanolammoniumacetate as a concentrate 1 6 mg of DHAS was weighed ad 10 ml of distilled water, and the same amount of AS From this 4 mM solution 0 4 mM was diluted with distilled water for each day A stock solution of 4 mM was also made of OS by diluting the estrone sulphate solution with distilled water

(3) Chloroform (Merck *p.A*), *pro analysi* chloroform was used undistilled

(4) Methylene blue reagent 125 mg of methylene blue (Methylenblau Geigy), 25 g of anhydrous Na_2SO_4 and 10 ml of concentrated sulphuric acid (Merck *p.A*) was dissolved in a small amount of water and diluted ad 1000 ml

(5) Alcohol Technical 96 per cent ethanol (Rajamäki factories) was diluted with distilled water to 75 per cent or used as such

Determination of activity

Into round bottomed centrifuge tubes was pipetted 0 2 ml of TRIS buffer and 0 4 ml of aqueous solution of steroid sulphate After preincubation for 5 min at + 37°C, 0 2 ml of homogenate was added

to the tubes The incubation time was 6 hours for DHAS and 3 hours for OS when rats were in question 3 hours for DHAS and 1 hour for OS when homogenates of human placenta were studied and 20 hours for all steroids when tissues of human fetus were analysed The tubes were stoppered to prevent evaporation The reaction was stopped and the proteins precipitated by adding 5 ml of 96 per cent ethanol and the tubes were allowed *to stand for 15—20 min The sediment* was centrifuged and the clear liquid decanted Five ml of the clear liquid was pipetted into Hagedorn tubes in which it was evaporated dry in a boiling water bath Each tube was removed from the water bath after it had been evaporated dry and was cooled in running cold water After the cooling 2 ml of methylene blue reagent and 5 ml of chloroform were added to the tubes They were shaken for 30 sec by the lateral shaking method Because an emulsion sometimes forms the samples were always centrifuged *The topmost layer of methylene blue was sucked* away as carefully as possible by Pasteur pipette Two ml of the chloroform phase was *pipetted into 10 ml of 75 per cent ethanol and the tubes were* shaken carefully The intensity of the colour was measured at wave lenght of 663 mμ against a blank using a light cell suitable for this wave length All the analyses were made in duplicate

The control for the steroid sulphate that failed to hydrolyse was always performed again as a duplicate or triplicate determination It was made in exactly the same way as the actual test except that the homogenate was added just before precipitation after incubation

The enzyme unit and its calculation

The splitting of steroid sulphates was calculated in μmoles of hydrolysed steroid sulphate per gram of tissue and hour and multiplied by 10^2 or in μmoles per milligram of nitrogen and per hour

The number of units (U_W) per wet weight was obtained by multiplying percentage number of splitting with *100 k in which k = 0 0133* for rat tissue that has hydrolysed DHAS 0 533 for placenta that has hydrolysed DHAS and 2 67 for OS

For nitrogen the number of units (U_N) was obtained in a corresponding way by multiplying with k the ratio of percentage number of splitting to mg of nitrogen in ml of 10 per cent homogenate

C Statistical calculations

The mean error of the difference of two independent determinations is calculated from formula

$$(1) \qquad s\{x' - x''\} = \sqrt{\frac{1}{n} \sum_{i=1}^{n} (x_i' - x_i'')^2}$$

in which n is the number of pairs of determinations The mean error of a single determination is thus

$$(2) \qquad s\{x\} = s\{x'\} = s\{x''\} = \frac{s\{x' - x''\}}{\sqrt{2}}$$

For the calculation of the mean error

$$(3) \qquad s\{\bar{x}\} = \frac{s\{x\}}{\sqrt{n}}$$

of the mean

$$(4) \qquad \bar{x} = \frac{1}{n} \sum_{i=1}^{k} n_i x_i$$

the standard deviation $s\{x\}$ is calculated from the formula

$$(5) \qquad s^2\{x\} = \frac{1}{n-1} \sum_{i=1}^{k} n_i (x_i - \bar{x})^2$$

in which x_i is the centre of class i, n_i its frequency, n the total frequency and k the number of classes

The confidence limits of the difference between the mean of the populations of the quantities x and y $(\mu_x - \mu_y)$ at risk level p are

$$(6) \qquad \left(\bar{x} - \bar{y} - t_p \, s \sqrt{\frac{1}{n_x} + \frac{1}{n_y}}, \; \bar{x} - \bar{y} + t_p \, s \sqrt{\frac{1}{n_x} + \frac{1}{n_y}}\right)$$

in which n_x and n_y are the total frequencies of the sampling of x and y

$$(7) \qquad s^2 = \frac{(n_x - 1)\, s^2\{x\} + (n_y - 1)\, s^2\{y\}}{n_x + n_y - 2}$$

and t_p is obtained from the t-distribution table the degrees of freedom being $n_x + n_y - 2$

The regression line of y in regard to x is calculated from the formula

to the tubes The incubation time was 6 hours for DHAS and 3 hours for OS when rats were in question 3 hours for DHAS and 1 hour for OS when homogenates of human placenta were studied and 20 hours for all steroids when tissues of human fetus were analysed The tubes were stoppered to prevent evaporation The reaction was stopped and the proteins precipitated by adding 5 ml of 96 per cent ethanol and the tubes were allowed to stand for 15—20 min The sediment was centrifuged and the clear liquid decanted Five ml of the clear liquid was pipetted into Hagedorn tubes in which it was evaporated dry in a boiling water bath Each tube was removed from the water bath after it had been evaporated dry and was cooled in running cold water After the cooling 2 ml of methylene blue reagent and 5 ml of chloroform were added to the tubes They were shaken for 30 sec by the lateral shaking method Because an emulsion sometimes forms the samples were always centrifuged The topmost layer of methylene blue was sucked away as carefully as possible by Pasteur pipette Two ml of the chloroform phase was pipetted into 10 ml of 75 per cent ethanol and the tubes were shaken carefully The intensity of the colour was measured at wave lenght of 663 mμ against a blank using a light cell suitable for this wave length All the analyses were made in duplicate

The control for the steroid sulphate that failed to hydrolyse was always performed again as a duplicate or triplicate determination It was made in exactly the same way as the actual test except that the homogenate was added just before precipitation after incubation

The enzyme unit and its calculation

The splitting of steroid sulphates was calculated in μmoles of hydrolysed steroid sulphate per gram of tissue and hour and multiplied by 10' or in μmoles per milligram of nitrogen and per hour

The number of units (U_K) per wet weight was obtained by multiplying percentage number of splitting with 100 k in which k = 0 0133 for rat tissue that has hydrolysed DHAS 0 533 for placenta that has hydrolysed DHAS and 2 67 for OS

For nitrogen the number of units (U_N) was obtained in a corresponding way by multiplying with k the ratio of percentage number of splitting to mg of nitrogen in ml of 10 per cent homogenate

A Arylsulphatase

Homogenate

The homogenate was made in distilled water because, as has already been mentioned several anions and cations have activating and inhibiting effects Since it was impossible to standardise the homogenisation completely, enzyme activity was studied in the same homogenate at the different phases of homogenisation The results given in Table IV, are the arithmetic means of three determination of the enzyme activity of 1 per cent rat homogenate

Table IV Effect of homogenisation degree on the enzyme activity

Degree of homogenisation	μg of liberated p-nitrophenol	Range
very little	13 3	13 0—13.5
crude	17 7	15.2—21 7
normal	17 2	16 2—18.2
prolonged	15 7	15 0—16 0

It was possible also to make a rough microscopical classification into the four degrees of homogenisation used in the present work.

Activity was slightly lower in the very crude homogenates than in the others Similarly, very prolonged homogenisation can result in inactivation principally thermal

The method of homogenisation employed in the present work can thus be regarded as suitable for the aims involved The wet weight percentage of the homogenate used is determined partly by the enzyme activity of the tissue and in the present investigation especially by the small size of the tissues When crude homogenates are used, 0 5—2 wet wt per cent is the range most commonly employed in enzymology

Inhibition often occurs in higher concentrations In the reaction conditions of the present work, the relation between enzymatic activity and percentage of homogenate was fairly linear from 0 25 to 2 per cent

(8) $$y - \bar{y} = b_{yx}(x - \bar{x})$$
where the regression coefficient of y on x

(9) $$b_{yx} = r \frac{s_y}{s_x}$$
in which s_x and s_y are the standard deviations of x and y and

(10) $$r = \frac{s_{xy}}{s_x s_y}$$
and in which further

(11) $$s_{xy} = \frac{1}{n-1} \sum_{i=1}^{k} \sum_{j=1}^{l} n_j (x_i - \bar{x})(y_j - \bar{y})$$

The confidence limits of the regression coefficient β_{yx} of the population at risk level p are

(12) $$b_{yx} \mp t_p \frac{s_y \sqrt{1-r^2}}{s_x \sqrt{n-2}}$$

while the number of degrees of freedom is n — 2. They are always calculated at risk level $p = 0.05$ and entered in the above form.

To study the significance of the difference between the correlation coefficients the following calculation was made

(13) $$z = \frac{1}{2} \ln \frac{1+r}{1-r}$$
the distribution of which is approximately normal with variance

(14) $$s^2\{z\} = \frac{1}{n-3}$$
and further

(15) $$\lambda = \frac{z_1 - z_2}{s\{z_1 - z_2\}}$$
in which

(16) $$s\{z_1 - z_2\} = \sqrt{s^2\{z_1\} + s^2\{z_2\}}$$
and the λ_p corresponding to risk level p is obtained from the normal distribution table.

The mean, the difference between two means and difference of the regression or correlation coefficients is termined significant if $0.01 < p < 0.05$, highly significant if $0.001 < p < 0.01$ and very highly significant if $p < 0.001$.

no significant error arise in normal practice from the poor capacity of the buffer

Optimum pH of the different tissues

The table compiled by DODGSON and SPENCER (1957b) shows a fairly great variety of pH optima for arylsulphatase This is due to a great extent to the large number of substrates and enzyme sources used the different buffers used and to the type of purified or unpurified fraction (fractions) measured

The optimum pH of rat liver has been given as 6 12 (HUGGINS and SMITH 1947) and 6 6 (ABBOT and EAST 1949) in acetate buffer using crude homogenate and NPS substrate The optimum pH of fraction C was 7 0 (DODGSON and THOMAS 1955) in 0 5 N acetate buffer with NPS substrate that of fraction A 5 8 in similar conditions and of fraction B 6 2

The literature gives no optimum pH values for crude homogenates of human tissue in acetate buffer with NPS as the substrate The optimum pH of fraction C of human liver is 7 3 and of fraction A 6 2 (DODGSON and WYNN 1956)

The optimum pH of rat liver kidney and intestine were studied in the reaction conditions of the present work The value obtained for liver and intestine was c 7 0 for the kidneys c 5 8 (Fig 3) The curves

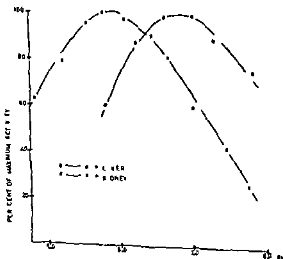


Fig 3 Effect of pH on the activity of arylsulphatase in rat liver and kidney The homogenate used for the liver was 1 and for the kidney 0 5 per cent wet wt. The substrate was 0 001 M NPS in acetate buffer

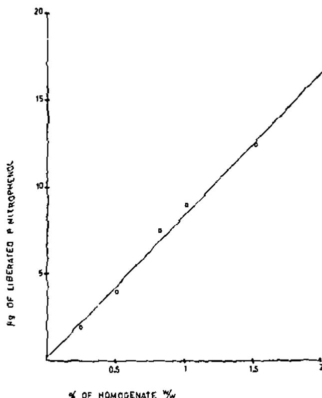


Fig 2 The effect of the homogenate percentage used on the hydrolysis of the substrate Rat liver in acetate buffer, pH 7 0, 0 001 M NPS

(Fig 2) If the homogenate percentage exceeds 3 no reliable results are obtained because of restrictions caused by the precipitation method and obvious protein inhibition

Buffer

The effective range of acetate buffer ends around pH 6 Thus it was not an effective buffer for pH 7 3 and 7 0, the most commonly employed values in the present work The literature contains some examples however, of the use of acetate solution in arylsulphatase determination even at high pH values like these (DODGSON and SPENCER 1957a DODGSON, SPENCER and THOMAS 1953)

The present author checked the pH changes at the beginning and end of incubation It might be expected that the products of hydrolysis originating in an enzymatic reaction are acid or basic, and that this would change the pH of the reaction milieu decisively during incubation It appeared, however, that although p nitrophenol was always liberated in amounts up to 30 μg in the reaction, the pH of the incubation solution did not change noticeably from 7 3 This shows that

kidney and lung is perhaps somewhat lower, 7 1, as can be seen from the curve obtained These tissues were measured 6 times each at pH 7 3 and 7 1, pH 7 1 gave slightly higher results (c 5 per cent) The result cannot be regarded as conclusive, it can only be said that the optimum pH is between 7 1 and 7 3 In practice however, pH 7 1 was used for these tissues

Substrate concentration

Substrate concentrations of 0 001 and 0 0005 M were used for rat liver in the literature in investigations using crude homogenates In the investigation of HUGGINS and SMITH (1947) the optimal concentration obtained was 0 003 M The optimal substrate concentration of fraction C of rat liver was 0 006 for NPS, and for fractions A and B 0 12 M In the present work, 0 001 M was selected as the substrate concentration for practical reasons Its blank value is also considerably smaller

The optimum substrate concentration for NPS when crude homogenate of human tissue is used has not been determined earlier For arylsulphatase fraction A of human liver the optimum is 0 07 M, of fraction C 0 008 M Substrate concentration optima were determined in the present work for the liver and lung of the fetus The same value was arrived at for both of them 0 008 M, and this is the value given in the literature for fraction C The results is shown in Fig 5

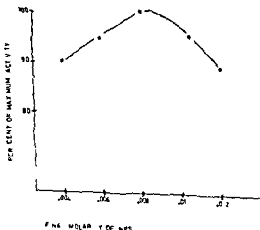


Fig 5 The effect of the substrate concentration on the reaction velocity Lung homogenate of human fetus (3 wet wt per cent) NPS substrate pH 7 1 acetate buffer

in this work were plotted from the mean values of triple determinations and each triple determination was performed three times The investigation of RUTENBURG and SELIGMAN (1956) is worth mentioning in this connection These workers, using 6 benzoyl 2 naphthyl sulphate as substrate, obtained a more alkaline optimum pH than 6 4 (the highest pH they tried) for rat liver and the same value as above, i e 5 8, as the optimum pH for kidney

The determination of the optimum pH in the acid milieu was complicated by the method of precipitation used in the present investigation 0 8 ml of 0 5 N NaOH is incapable of alkalising an acetate buffer of under pH 6 2 sufficiently to produce the maximum colour in the liberated p nitrophenol In the actual test analyses therefore 0 63 N NaOH was used, and this was suitable In the determination of the pH curves this source of error was taken into consideration by varying the normality of NaOH, but an excess of NaOH in these conditions can easily produce turbidity in the tubes after precipitation

Optimum pH values were determined for the following human fetal tissues liver, kidney, pancreas, intestines, lung and placenta The optimum obtained for liver, pancreas intestines and placenta was 7 3 Fig 4 shows the results for liver and kidney The optimum pH of

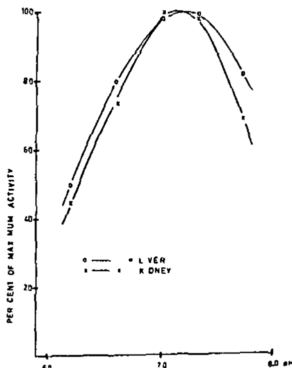


Fig 4 pH activity curves for homogenates of liver and kidney of human fetus 0 005 M NPS substrate in acetate buffer

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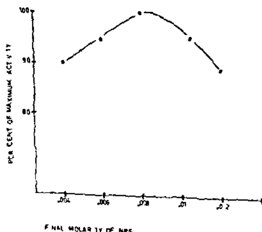


Fig 5 The effect of the substrate concentration on the reaction velocity Lung homogenate of human fetus (3 wet wt per cent) NPS substrate pH 7 1 acetate buffer

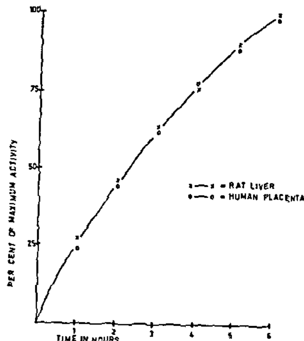


Fig 6 The effect of incubation time on the reaction velocity Homogenates of rat liver and human placenta (1 wet wt per cent) For rat, 0 001 M NPS, pH 7 0, for man, 0 005 M NPS, pH 7 3 Acetate buffer

Incubation temperature and time

The optimum incubation temperature for arylsulphatase has been given as 60° C (ABBOT and EAST 1949) RUTENBURG and SELIGMAN (1956) observed that the reaction was most linear at 37° C At 60° C the beginning of the reaction was rapid, but retardation occurred later on The temperature generally used is in fact 37° C and this was adopted for the present investigation

The thermostat of the water bath kept the water temperature at 37° C with an accuracy of $\pm$ 0 5° The samples had generally warmed up already at room temperature and were preincubated for 3—5 min to achieve a constant reaction velocity throughout the test

Enzyme activity as the function of time need not be linear over a very wide range The curves obtained for rat liver show a relatively good but not exact linearity up to c 3 hours after which the reaction slows down (Fig 6)

Fig 6 shows the experiment with human placenta The incubation time used was affected by the shape of the function of time curve and also by the relative enzyme content of the tissues An endeavour was made to obtain an adequate amount of liberated p nitrophenol for colorimetric determination by varying the incubation time The incubation times shown in Table 3 were arrived at in this way

Precipitation of the proteins

If protein precipitation is a two-phase procedure as in the present work the first chemical added must stop the reaction If zinc sulphate is added first 0 5 ml of 10 per cent solution the enzymatic reaction is stopped almost completely a c 90 per cent inhibition occurs DODGSON and POWELL (1959) observed the inhibitory effect of zinc chloride also However it was found that the reaction did cease completely when sodium hydroxide was added This practice was followed It involves the possibility of NPS splitting non-enzymatically in the alkaline milieu (DODGSON and SPENCER 1954) The serum of mammals contains a thermostable and non-dialysible factor which splits NPS non-enzymatically in a reaction This is dependent on the strength of the alkalinity and on the temperature If the amount of p nitrophenol is determined immediately after the base has been and again 6 hours later no notable differences will be found in these test conditions However the proteins were precipitated by adding zinc sulphate immediately at least not later than 1 hour after the addition of sodium hydroxide

Another risk involved in protein precipitation is that the liberated phenol in this case p nitrophenol might be adsorbed into the precipitate If $ZnSO_4$—NaOH precipitation is used by adding them in the ratio employed in this work 100 per cent recovery of p-nitrophenol is obtained This can be seen from the calibration curves obtained when water was substituted for the homogenate used in the experiment and when the homogenate was added in the usual way The straight lines obtained converge

The precipitation used in the present work is not effective if there is much protein The highest homogenate percentage used (3 per cent) precipitates well without giving a disturbingly high blank value The solution remains turbid if the percentage of the homogenate used exceeds 5

When zinc sulphate was added to alkaline solution in the ratio used here a precipitate originated which was first flocculent then powdery Filtering was performed immediately after adding $ZnSO_4$ because the powdery precipitate that had formed sometimes adsorbed p nitrophenol and produced turbidity in the solutions to be measured

The colour stability of the filtered green solution was good Storage for 24 hours did not effect the intensity

Metabolisation of the liberated p nitrophenol and its maximum absorption

Caution is necessary in using NPS as the substrate for the fresh homogenate of rat liver since rat liver may metabolise p nitrophenol liberated in an enzymatic reaction (DODGSON and SPENCER 1953 i) On incubating rat liver homogenate and this phenol in acetate buffer these workers obtained only partial recovery p Nitrophenol solution thus incubated showed a small peak on the absorption curve at 290—295 mμ and the metabolisation product was identified by paper chromatography as p aminophenol Quantitative analysis showed that it made up for the deficit of the recovery test The rat strain used by DODGSON and SPENCER was a Medical Research Council Strain which occasionally included albinos It is to be noted that this phenomenon was not observed in rat intestine nor was the mouse liver able to carry out this metabolisation These phenomena are however fairly *type and strain specific as is shown by the arylsulphatase literature* as a whole on many points concerning enzyme determination methods

Thus phenomenon was checked occasionally for the rat strain used *in the present work 0 5 ml of 1 per cent rat liver homogenate in 1 5 ml* of acetate buffer, pH 7 0 was incubated with 0 5 ml of p nitrophenol aqueous solution 15—30 μg of phenol per ml for 3 hours after which the proteins were precipitated in the usual way and the intensity of the colour measured at 402 mμ In the control determinations the p nitrophenol was added to the incubation mixture just before precipitation keeping the other components constant in other control experiments the homogenate was replaced by distilled water Livers of rat fetuses newborn rats and rats aged 1 month and adult rats of both sexes were used in the experiment Three parallel determinations were made for the controls too and the experiment was repeated a couple of times in the course of the test series No metabolisation of p nitrophenol in the form of incomplete recovery was observed

Furthermore pure p aminophenol which is assumed to be a metabolite was re crystallised and decolorised by means of active carbon and the absorption curves of this p aminophenol and incubated p-*nitrophenol were compared* No peak was observed in the latter at the absorption maximum of p aminophenol

The ability of the liver or kidney of human fetus to metabolise p nitrophenol was studied in the same way as for rat No metabolisation was established in these cases either

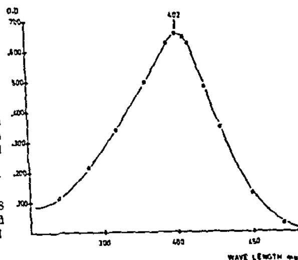


Fig 7 The absorption curve of p-nitrophenol in alkaline solution. 1 5 ml of acetate buffer, pH 7.3 0 5 ml of distilled water 0.5 ml of aqueous solution of p-nitrophenol 0 8 ml of 0 5 N NaOH and 0 5 ml of $ZnSO_4$, filtered

The colour of p nitrophenol has been measured at different wave lengths (HUGGINS and SMITH 1947, DODGSON and SPENCER 1953 a) The absorption maximum varied slightly with the milieu. In the present work, the absorption maximum of p-nitrophenol was established as 402 mμ (Fig 7)

Stability of the enzyme

The enzyme activity decreased to c 75 per cent in an acetone suspension of marine molluscs when the solution was preincubated for 1 hour (DODGSON, LEWIS and SPENCER 1953) Activity did not decrease in a few hours at 0°C It is generally held that arylsulphatase retains its stability long at —15°C (DODGSON SPENCER and WYNN 1956) Since there is no detailed information on this question which is an important one for the practical execution of the present work, the stability of the enzyme activity of human and rat homogenates was studied after keeping them in different concentrations for a day, a week and a month at room temperature (c + 20°C), at + 4°C and at — 15°C The results are shown in Table V Arylsulphatase was less well preserved in a weak solution which is a common finding When kept at — 15°C no notable changes in activity occurred in 1 per cent homogenate At + 20°C and + 4°C on the other hand, the enzyme did not retain its activity for long and the later rise at room temperature has its natural explanation in the presence of this enzyme in many bacteria

Table V Stability of arylsulphatase in homogenate of human fetus and rat

Duration of storage	Storage temperature	0 2 % human liver	1 % human liver	1 % human kidney	1 % rat liver
fresh	—	100	100	100	100
1 day	+20	2	25	62	32
"	+ 4	26	79	82	92
"	—15	55	110	90	93
1 week	+20	0	1	22	76*
"	+ 4	5	41	70	32
"	—15	35	104	85	72
1 month	+20	10*	2*	51*	—
"	+ 4	11	25	25	—
"	—15	38	81	78	60

* marked putrefaction

Precision

It have been calculated the error of single determination for to study the arylsulphatase method

Table VI Error of a single determination of the method

Ranges of μg liberated p-nitrophenol	0—2 5	2 5—5 0	5 0—10 0	10 0—20 0	over 20	all
n	57	57	55	56	56	281
$\bar{x}$	1 12	3 96	6 89	14 05	29 77	8 79
s {x}	0 226	0 53	0 60	1 16	1 83	0 90
%	20 2	13 3	8 8	8 3	6 1	10 2

n = number of determinations in each class
$\bar{x}$ = mean of the class
s {x} = standard error of its single determination
% = s {x} in per cent of $\bar{x}$

Table VI shows that the object in splitting NPS should be the highest percentage possible within the limits imposed by enzymological considerations proper The mean accuracy of the parallel determinations in the present work was the same as obtained by KIRK and DYRBYE (1956) in a similar modification

B. Hydrolysis of steroid sulphates

Regulation of the splitting

The technical procedure for making homogenate for hydrolysing steroid sulphates was the same as for arylsulphatase. The homogenate percentages and incubation times given in Table VII were selected in order to obtain suitable percentages in hydrolysis of steroid sulphate.

Table VII. Milieu used for the splitting of steroid sulphates. pH 7.8 and 0.2 mM steroid sulphate

Type of tissue	Incubation time hours DHAS	OS	AS	Homogenate percentage DHAS	OS	AS
rat liver	6	6	20	10	1	10
human placenta	3	3	20	5	1	10
tissues of human fetus	20	20	20	10	10	10

The aim was to achieve a splitting degree of c. 20 per cent. This would not change the substrate concentration to any appreciable extent but would permit accurate results (cf. Table IX).

Purity

Unless the synthetic detergent used to wash the tubes is carefully rinsed out, a nonspecific addition of methylene blue or a complex of this dye and some foreign agent may take place in the chloroform phase and render the results entirely unreliable.

Reaction conditions

A substrate concentration of 0.2 mM has been used in the literature for both mammals and *Patella* enzyme. It was considered suitable also in the present work since there was nothing to suggest the contrary. There is no mention in the literature of the possible inhibition of TRIS buffer. The pH value used 7.8 has been suggested as the optimum (Roy 1957). In the few determinations which were made at pH 6.0 and 8.5 using DHAS as the substrate, activity was observed to be smaller than at pH 7.8. Thus by and large this was the optimum also in the present investigation.

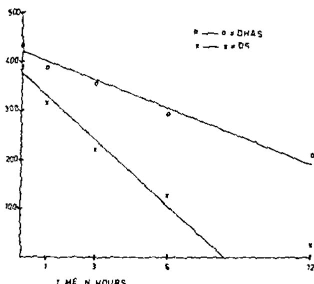


Fig 8 Splitting of DHAS and OS by 10 and 1 wet wt. per cent rat liver homogenate, as a function of time 0.2 mM steroid sulphate and pH 7.8 in TRIS buffer

Reaction as a function of time

A 6 hour reaction time is generally to be considered long For this reason, the splitting of DHAS and OS with rat liver homogenate was studied as the function of time The results are shown in Fig 8 For these conditions, the linearity can be considered relatively good A decrease from the original substrate concentration down to 40 per cent does not seem to have any great effect on linearity

Absorption spectrum of the complex and calibration

The maximum absorption of the steroid sulphate methylene blue complex has been given as 700 mμ (Roy 1957) Using the methylene blue of different manufacturers and different steroid sulphates 663 mμ was obtained as the maximum absorption of the steroid sulphate methylene blue complex in alcohol solution (Fig 9) Lyon Bullbrook and Corner (1960) later measured complexes at 655 mμ The value obtained in this work as the absorption maximum for the complex in a non alcohol solution was 658 mμ The absorption maximum of aqueous solution of pure methylene blue measured against the solvent was 667 mμ

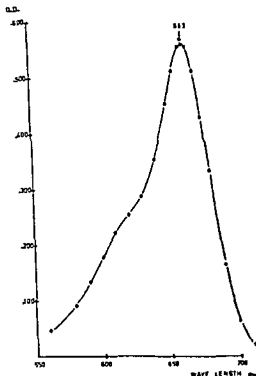


Fig 9 Absorption curve of the complex formed by methylene blue and steroid sulphates, in chloroform alcohol solution

The formation of the complex by methylene blue and steroid sulphate and its extraction in chloroform were linear in the present study The calibration curves obtained for DHAS OS and AS are given in Fig 10

Stability of the hydrolysing ability of steroid sulphates

Rat liver homogenate was stored at a concentration of 10 per cent for DHAS and 1 per cent for OS The times of storage of each sample temperatures and their hydrolysing ability after a given period are shown in Table VIII

The enzymes studied appear to be relatively stable especially when kept in higher concentrations and at —15°C at room temperature on the other hand natural inactivation occurred

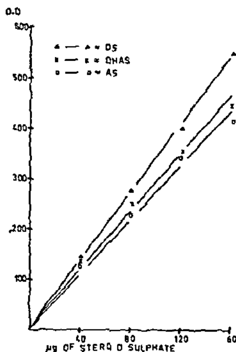


Fig 10 Calibration curves for DHAS, OS and AS

Table VIII Stability of the splitting capacity of steroid sulphates Rat liver The results are given in per cent of steroid sulphate hydrolysed.

Durat on of storage	Storage temperature	DHAS	OS
fresh	—	21 9	34 3
1 day	+20	16 9	26 5
	+ 4	21 7	25 4
	−15	21 7	25 6
1 week	+20	20 2	1 4
	+ 4	21 7	34 7
	−15	16 9	30 7
1 month	+20	−6 6	4 4
	+ 4	4 5	16 2
	−15	14 7	39 4

Precision

It have been calculated the error of single determination for to study the precision of steroid sulphate method

Table IX Error of a single determination of the method.

Ranges (% of split steroid sulphate)	0—10	10—20	20—30	over 30	Total
DHAS					
n	26	12	30	11	79
$\bar{x}$	5.4	14.7	24.2	33.5	17.9
$s\{x\}$	5.0	4.6	5.9	4.9	5.3
%	94	31	24	15	30
OS					
n	22	24	18	15	79
$\bar{x}$	5.4	15.0	25.8	39.7	3.9
$s\{x\}$	4.7	3.7	5.1	2.9	19.5
%	86.1	25	20	7	20

For the method to give fairly reliable results the splitting percentage should be at least 25 In order to achieve this the main factors varied were incubation time and hogomenate percentage

RESULTS

A Arylsulphatase

1 Human arylsulphatase

Localisation in different organs

The arithmetic means and the enzyme activities of the different organs of the fetus material as a whole are shown in Table X The table also reveals the number of the determinations made per wet weight on the one hand and per nitrogen content on the other

The enzyme was studied in human fetus in 26 different organs or secreta The first observation made was that man shows relatively high arylsulphatase activity already in the fetal period The localisation of the enzyme between the different organs was clear It was most profuse in the liver, intestines, pancreas and kidney In the few experiments to determine the arylsulphatase activity of the gallbladder it was found closest to that of the pancreas The highest concentrations in the intest

Table X. The amount and localisation of arylsulphatase in human fetal organ. Crown heel length of fetus 0.5—33 cm

Tissue	Calculation per wet weight (U_w)		Calculation per tissue nitrogen content (U_N)	
	$\bar{x} \pm s\{\bar{x}\}$ (n)	range	$\bar{x} \pm s\{\bar{x}\}$ (n)	range
liver	1260±71 (64)	540—3220	770±50 (55)	270—2300
ileum	675±50 (40)	186—1620	410±36 (39)	80—1470
jejenum	600±45 (41)	222—1380	390±31 (37)	160—820
colon	420±44 (41)	103—1400	194±12 (33)	65—520
duodenum	373±29 (43)	130—1080	310±32 (37)	75—750
intestines (smallest)	232±28 (19)	66—480	240±54 (8)	65—350
pancreas	186±17 (33)	60—400	134±12 (27)	55—240
kidney	124±8.9 (71)	3—300	118±8.1 (51)	16—300
lungs	40.3±3.6 (59)	3—133	47±3.7 (48)	5—96
stomach	37.6±2.6 (45)	6—102	29±3.1 (29)	6—93
brains	13.5±1.24 (54)	3—36	17.7±1.48 (48)	1—41
genitals	11.5±1.65 (29)	1—32	10.0±1.76 (22)	2—35
salivary gland	9.9±2.51 (26)	1—66	10.3±1.68 (21)	1—37
adrenal gland	8.9±0.89 (59)	1—24	6.5±0.57 (42)	1—16
heart	7.1±0.94 (48)	1—31	5.0±0.70 (39)	1—21
thyroid gland	6.0±0.92 (35)	0—13	5.1±0.71 (34)	0—16
skeletal muscle	5.8±0.71 (27)	0—12	5.3±0.67 (21)	0—10
thymus	4.8±0.65 (35)	0—14	3.6±0.54 (29)	0—12
spleen	4.4±0.41 (41)	0—13	2.3±0.44 (28)	0—11

ines were around the middle of it, in the jejunum and in the ileum. The concentration was higher in the latter, but the difference between the two parts was not statistically significant. The colon and the duodenum were nearly equally rich in the enzyme. There was relatively little activity in the upper part of the digestive tract, the stomach. Pulmonary tissue contained less enzyme than the poorest of the tissues above, the kidney, but its activity was greater than that observed in the brains, especially in larger fetuses. No significant differences were observed in the few cases in which the different parts of the brains were analysed. The genitals revealed a distinctly demonstrable enzyme content in the fetal period. Activity figures of the same magnitude were obtained from the oviduct, the testis, the uterus and the genitals in general. Weak activity was established in the cardiac muscle, and the skeletal muscle

had hardly any enzyme With the exception of the pancreas the glandular tissues do not possess much arylsulphatase The highest activities were established in the sexual glands of fetuses and the salivary glands (gl parotis) of larger fetuses The adrenal gland also displayed weak activity It was impossible to establish any definite enzyme activity towards NPS in the thyroid gland, the spleen and the thymus The values recorded for them approximated to a technical potential error Nor was any activity established either in the bone marrow or in the bones themselves No activity was present in the walls of the three urinary bladders examined

Of the secreta, meconium had a fairly large amount of arylsulphatase No activity was established in the urine or amniotic fluid The method is not suitable for examination of the blood because of its high protein content

Arylsulphatase in the organs during the organogenetic period of human fetus

Liver

The arylsulphatase activity of 64 fetus livers was studied and the nitrogen content of the liver homogenate determined in 55 of them The crown heel length of the fetuses ranged *from 2 0 to 33 0* cm, mean 11 8 cm The mean enzyme activity established was 1260 ± 70 U_w and 770 ± 50 U_N The ranges were 540—3220 U_w and 270—3200 U_N The regression between enzyme activity and length is shown in Fig 11 where the results were calculated per wet weight No statistically significant regression was observed between length and activity with either method of calculation

Alimentary canal

(a) *Alimentary canal as a whole* It was considered impossible in 19 fetuses under 9 *cm in* length to distinguish with sufficient accuracy between the different parts of the alimentary canal, and enzyme activity was consequently determined *from the* whole Because of the small size of these fetuses tissue nitrogen was determined in 8 *cases only* The

U_w	11	17	15	14	6	0	1	64
3250			1		1			2
2750								0
2250	1			2				3
1750	1	2	4	3	2			12
1250	4	8	x5	3	1		1	22
750	5	7	5	6	2			25
y / x	2.5	7.5	12.5	17.5	22.5	27.5	32.5 cm	

Fig 11 The regression of the arylsulphatase activity of human liver to fetal length, calculation per wet weight. $p > 0.05$

length of the fetuses ranged from 2.5 to 7.9 cm, mean 5.5 cm The mean enzyme activity of the alimentary canal as a whole was 232 ± 28 U_w and 240 ± 54 U_N The regression as regards fetus size was statistically significant per wet weight There was no regression as regards nitrogen, but the series was rather small in this group, only 8 fetuses

(b) *Stomach* The material consisted of 45 stomach walls in 29 of which tissue nitrogen was determined The fetus length ranged from 8.5 to 33 cm, mean 13.9 cm The smallest arylsulphatase activity in the digestive tract was that of the stomach an average of 37.6 ± 2.6 U_w and 29 ± 3.1 U_N The ranges were 6—102 U_w and 6—96 U_N The regression between growth and arylsulphatase content was statistically highly significant in the stomach wall calculated per wet weight ($r = 0.459 \pm 0.118$ $p = 0.01$), activity rose 1.2 ∓ 0.64 U_w/cm In terms of tissue nitrogen, however, it was impossible to establish the same correlation and the rise was thus ostensible

(c) *Duodenum* The duodenum of 43 fetuses was studied and in 37

cases a tissue nitrogen determination was performed The fetus length was 6 5—28 5 cm mean 14 9 cm The enzyme activity established was considerably greater than the comparable activity in the stomach wall 373 U_w and 310 U_N, while the standard deviations of their means were 29 U_w and 32 U_N The ranges were 130—1080 U_w and 75—750 U_N There was no regression as regards length

(d) *Jejunum* Forty one jejunums were examined and 37 type analyses made of them The length of the fetuses was 7 5—28 5 cm mean 15 2 cm The arylsulphatase activity was 600 ± 45 U_w and 390 ± 31 U_N The regression between enzyme activity and the fetal length was not statistically significant

(e) *Ileum* The arylsulphatase activity of 40 fetus ileums was determined and for 39 of them the tissue nitrogen was determined The length of the fetuses was also 7 5—28 5 cm, mean 15 6 cm The enzyme content was 675 ± 50 U_w and 410 ± 36 U_N The activity of the ileum was thus somewhat greater than the jejunum activity, but the difference was not statistically significant The regression between enzyme activity and fetal length was not statistically significant

(f) *Colon* The material consisted of 41 fetus colons and 33 of them were analysed for tissue nitrogen The fetuses were the same as those used in determining jejunal enzyme activity The contents were 420 ± 44 U_w and 194 ± 12 U_N The arylsulphatase activity of the colon was statistically very highly significantly lower than in the jejunum and ileum No regression in relation to fetal length was established in this part of the alimentary canal either

Pancreas

The pancreas of 33 fetuses was examined Tissue nitrogen was determined in 27 of them The crown heel length of the fetuses was 8 5—33 0 cm mean 16 4 cm The average enzyme activity in the pancreas was 186 ± 17 U_w and 134 ± 12 U_N, limit values 60—400 U_w and 55—240 U_N No significant increase was established in enzyme activity with the growth of the fetus

Kidney

The kidney of 71 fetuses was analysed and the tissue nitrogen determined in 51 of them The fetus length was 2 0—33 0 cm mean 12 4 cm Enzyme activities of the following magnitude were established in the

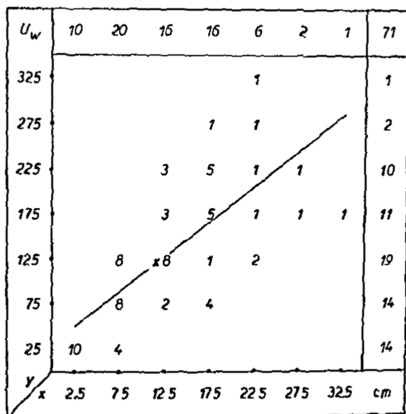


Fig 12 Regression of the arylsulphatase activity of human kidney to fetal length, calculation per wet weight $p < 0\ 001$.

kidney 124 ± 8 9 U_w and 118 ± 8 1 U_N The limit values were 3—300 U_w and 16—300 U_N Enzyme activity increased statistically very highly significantly with fetal growth, per wet weight and per tissue nitrogen ($r = 0\ 713 \pm 0\ 058$, $p < 0\ 001$ calculated per wet weight and $r = 0\ 716 \pm 0\ 068$, $p < 0\ 001$ calculated per tissue nitrogen) The regression between activity and length is shown in Fig 12 The regression line gives the increase as 7 67 ∓ 0 908 U_w/cm and 6 44 ∓ 0 755 U_N/cm

Lung

The material included 59 fetuses and 48 nitrogen analyses were made of the lung homogenate The crown heel length of the fetuses was 2 5—28 5 cm, mean 11 4 cm The observed arylsulphatase activity varied from 3 to 130 U_w and 5—96 U_N, means 40 3 ± 3 6 U_w and 47 ± 3 7 U_N The arylsulphatase content increased both per wet weight

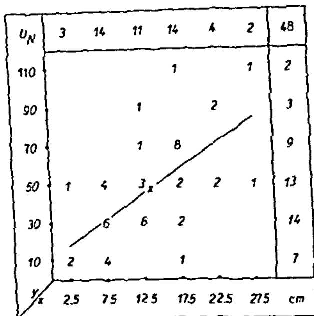


Fig 13 The regression of the arylsulphatase activity of human lung to fetal length calculation per nitrogen content, $p < 0.001$

and per tissue nitrogen content as the fetus developed The regression between activity and length was statistically very highly significant by both methods of calculation ($r = 0.60 \pm 0.082$ and $p < 0.001$ and $r = 0.618 \pm 0.089$ and $p < 0.001$ respectively) The values of the regression line per wet weight were $b_{yx} = 2.7 \mp 0.936$ U_w/cm and per tissue nitrogen 2.59 ∓ 0.977 U_N/cm The regression line for pulmonary enzyme activity and fetus length is given in Fig 13 The correlation coefficient for the enzyme activity of the lung and fetal length did not differ statistically significantly from that for the kidney in other words enzyme activity increased linearly as good in the lung and in the kidney during the period of fetal organogenesis studied

Brain

The brain showed some arylsulphatase activity though it was fairly small The material consisted of 54 fetuses for 48 of which the brain tissue nitrogen was determined Activity varied from 2.5 to 30.8 U_w

and 6 5 — 41 0 U_N, means 13 5 ± 1 24 U_w and 17 7 ± 1 48 U_N The regression as regards the length of the fetus was not significant No notable increase in activity occurred during the period studied

Other tissues

Enzyme activity was very small in tissues other than these tissues detailed above Such values as were obtained cannot be regarded as fully reliable quantitatively since they were relatively close to the blank values The results in these cases are therefore given in the form of 3 categories only, divided according to fetal length under 9 cm, 9—16 cm and over 16 cm These lengths were chosen as they correspond roughly to the limits between the third and fourth months of preg nancy

The results obtained are given in Tables XI and XII

Table XI The enzyme concentrations of some arylsulphatase poor tissues by fetal size groups, calculation per wet weight $\bar{x} \pm s\{\bar{x}\}$ (n)

Tissue	Under 9 cm	9—16 cm	Over 16 cm
genitals	10 8 (2)	9 4±1 65 (14)	14 1±2 8 (11)
salivary gland	2 2 (3)	7 7±1 16 (10)	14 3±5 6 (10)
adrenal gland	8 5±0 96 (18)	10 2±1 67 (23)	7 7±1 8 (15)
heart	7 5±1 52 (18)	8 7±1 89 (15)	4 6±1 14 (12)
thyroid gland	3 9±0 86 (4)	5 5±1 04 (16)	7 4±1 9 (12)
skeletal muscle	6 3 (2)	5 0±0 85 (16)	5 7±1 5 (7)
thymus	—	4 6±0 30 (20)	2 2±0 81 (12)
spleen	2 3±0 49 (8)	5 5±0 59 (17)	4 3±0 69 (14)

Table XII The enzyme concentrations of some arylsulphatase poor tissues by fetal size groups, calculation per tissue nitrogen content $\bar{x} \pm s\{\bar{x}\}$ (n)

Organ	Under 9 cm	9—16 cm	Over 16 cm
genitals	3 5 (1)	10 4±3 1 (10)	10 3±2 0 (11)
salivary gland	—	9 5±1 55 (11)	11 2±1 03 (10)
adrenal gland	8 1±1 3 (9)	6 3±0 77 (19)	5 7±1 03 (14)
heart	4 8±1 43 (14)	5 8±1 05 (15)	4 2±1 20 (10)
thyroid gland	2 3 (1)	9 5±0 93 (21)	1 6±1 19 (12)
skeletal muscle	6 3 (2)	5 0±0 87 (12)	5 7±1 34 (7)
thymus	—	4 6±0 78 (17)	2 2±0 57 (12)
spleen	—	2 8±0 73 (16)	1 4±0 34 (12)

Arylsulphatase was generally found during the fetal period in the genitals Activity was greater on the whole in large (over 16 cm) fetuses than in the other two categories but the difference was not statistically significant The salivary glands possessed the same amount of activity as the genitals In the thyroid gland and the cardiac muscle there was perhaps some activity but for the spleen and skeletal muscle the values obtained were below the lower limit of the method and no actual enzyme activity could be said to exist

2 Arylsulphatase in human placenta

Arylsulphatase activity of the placenta during the earlier fetal period

(a) Fetal side The enzyme activity on the fetal side was determined in 55 placentas The placentas belonged to fetuses with a crown heel length of 0 5—33 cm mean 12 1 cm The nitrogen content of the homogenate was analysed for 40 of them The mean arylsulphatase content was 191 ± 11 2 U_w and 183 ± 14 1 U_N The wet weight values gave a statistically significant regression in which b_{yx} = 3 86 ∓ 3 42 U_w/cm The correlation coefficient was 0 298 p = 0 05 The tissue nitrogen values however gave a regression that was not statistically significant

(b) Uterine side Samples were taken from the placentas of 50 fetuses with a crown heel length of 0 5—33 cm mean 11 9 cm The nitrogen content of 39 samples was determined. The activity observed was 171 ± 12 1 U_w and 156 ± 15 6 U_N The activity of arylsulphatase grew in the placenta also on the uterine side as the fetus grew (4 1 ∓ 3 57 U_w/cm r = 0 318 p = 0 05) The result was statistically significant

It was impossible here again to observe a significant increase in activity from the results calculated per nitrogen content

There was a statistically highly significant difference between the activity on the fetal and on the uterine side of the placenta in the results calculated per wet weight per tissue nitrogen there was only a tendency to greater activity on the fetal than on the maternal side

The samples taken from the maternal side showed a mean activity of 241 ± 15 1 U_w and 104 ± 5 2 U_N, those from the fetal side of 218 ± 17 U_w and 86 6 ± 6 90 U_N Taking each placenta separately, the difference between these two means was significant both per wet weight and per tissue nitrogen content ($p = 0\ 05$) There was more arylsulphatase on the mother s side The wet weight value for activity was greater in the placenta at the time of delivery than in the earlier fetal period The difference was statistically highly significant ($p < 0\ 01$) This was not the case with the values calculated per tissue nitrogen content, the situation was the reverse the difference highly significant

The series included fraternal twins whose placental activity was close to the mean for the series and near each other (256 and 280 U_w and 258 and 288 U_N) It also contained 2 placentas of subjects with mild pre-eclampsia in which the activity did not differ notably from the general level

3 Rat arylsulphatase during intra and extrauterine development

Intrauterine development

(a) Liver The enzyme content of the liver of 65 fetuses was analysed per wet weight The weight of the fetuses was 0 5—5 56 g mean 2 4 g The mean arylsulphatase activity in the liver of rat fetus was 16 0 ± 1 12 U_w There was a statistically very highly significant regression between the total weight of the fetus and activity i e 2 80 ∓ 1 14 U_w/g in which $r = 0\ 525$ and $p < 0\ 001$ The regression curve is shown in Fig 14

It was possible determine the tissue nitrogen content in the livers of 43 fetuses The total weight range in this group was 0 77—5 5 g mean weight 2 97 g The fetuses were a little larger as the homogenate from the smaller fetuses did not suffice for the nitrogen determination The mean activity was 9 7 ±0 27 U_N The regression was statistically highly significant even when calculated in this way an increase of 1 48 ∓ 1 08 U_N per gram ($r = 0\ 390$ $p < 0\ 01$) The increase in the

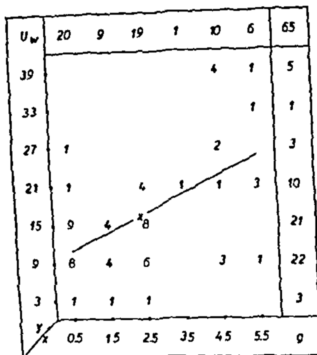


Fig 14. The regression of the arylsulphatase concentration of rat liver to fetal weight calculation per wet weight. $p < 0.001$

tissue nitrogen value was not as sharp as in the wet weight values

(b) Kidney Activity determinations per wet weight were performed for the kidneys of 49 fetuses of different sizes The fetal weight range was 0.77—5.51 g mean 2.84 g Owing to the small size of the kidneys of fetuses under 1.5 g the kidneys of several fetuses of the same mother were pooled for analysis and the mean fetal weight was entered as the size The arylsulphatase activity of rat kidney in the fetal period was thus assessed as 12.7 ± 0.97 U_w Growth in fetal size was accompanied by very highly significant increase in enzyme content 2.96 ∓ 1.01 U_w/g ($r = 0.619$ $p < 0.001$) The results are shown in Fig 15

The tissue nitrogen content was determined in 28 fetuses weighing 1.98—5.56 g mean 2.97 g i e only the largest individuals The mean activity was 7.62 ± 1.11 U_N These large fetuses revealed no statisti

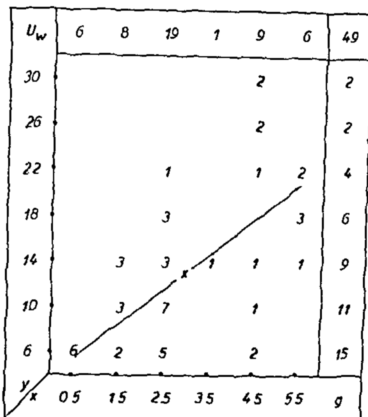


Fig 15 The regression of the arylsulphatase concentration of rat kidney to *fetal weight, calculation per wet weight. $p < 0\ 001$*

cally significant regression as regards size although the same tendency *was observable*

(c) Alimentary canal The enzyme activity of the alimentary canal of g 0 61—5 56 weighing fetuses was measured The mean weight was 2 26 g *enzyme activity 6 3 ± 0 31 The regression was 0 77 ∓ 0 32* U_w/g ($r = 0\ 522$ $p = 0\ 01$) The growth of activity with the increase in weight was thus statistically highly significant

The alimentary canals of 43 rat fetuses were analysed for tissue nitrogen The fetal weight range was 0 77—5 56 g mean 2 82 g The mean *enzyme content was 4 6 ± 0 25* U_N/g but the regression in regard to size was not statistically significant

Table XIII The arylsulphatase activity of rat liver, kidney and intestine by age groups

$\bar{x} \pm s\{\bar{x}\}(n)$

Age	Liver		Kidney		Intestine	
	(U_w)	(U_V)	(U_w)	(U_V)	(U_w)	(U_V)
fetus under 4 g	12 9±0 86 (49)	9 8±0 95 (43)	12 7±0 97 (49)	5 6±0 68 (16)	5 8±0 30 (46)	3 72±0 34 (39)
fetus over 4 g	24 6±3 3 (15)				7 5±0 67 (10)	
under 24 hours	26 1±1 75 (46)	11 9±0 87 (46)	32 5±2 2 (28)	17 7±1 93 (22)	8 1±0 60 (11)	3 9±0 32 (42)
1 month	70 3±3 8 (30)	24 9±1 59 (38)	73 1±8 1 (48)	22 0±0 67 (29)	7 2±0 71 (47)	2 4±0 22 (33)
3 ½ month	109±4 4 (51)	38 3±1 83 (43)	91 0±2 50 (55)	28 9±1 41 (12)	0 5±0 65 (52)	3 3±0 56 (40)

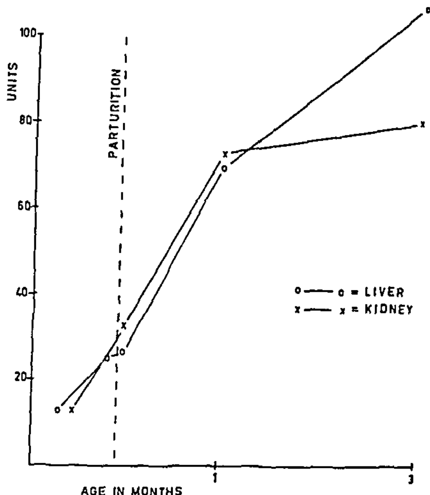


Fig 16 Rat arylsulphatase as a function of age in liver and kidney Substrate *0 001 M NPS*, acetate buffer, pH 7 0 for liver and 5 8 for kidney, and homogenate percentages 1 0 and 0 5 respectively μg of liberated p nitrophenol/100 mg wet wt /hr

Enzyme during extrauterine development

Arylsulphatase concentrations in different age groups

Table XIII summarises the activities by age groups The results for the liver and the kidney are presented graphically in Fig 16

(a) Newborn The liver of 46 newborn rats was studied and analysed for tissue nitrogen content Enzyme activity was 26 1 $\pm$ 1 7 U_w and 11 9 $\pm$ 0 87 U_N The kidney contained 32 5 $\pm$ 2 2 U_w and 17 7 $\pm$ 1 9 U_N

and the intestine 8.18 ± 0.60 U_w and 3.9 ± 0.32 U_N. Comparing the results with fetus enzyme concentrations in general the difference in the liver per wet weight was very highly significant activity being higher in the newborn. Making a similar comparison for the nitrogen unit values the increase was not statistically significant ($t = 1.75$) but the same tendency could be observed. Because of the small size of the fetuses tissue nitrogen determinations were possible only for the major fetuses (mean weight 2.97 ± 0.27 g against 2.40 ± 0.21 g) and besides this the activity rose very highly significantly per tissue nitrogen as the fetus grew.

In order to demonstrate possible changes in enzyme content at the moment of birth the liver arylsulphatase activity of fetuses weighing over 4 g was calculated. It was 24.6 ± 3.3 U_w (15 analyses). The concentration was fairly close to that established for newborn rats (26.1 ± 1.7 U_w); the difference was not significant.

The kidney arylsulphatase content of newborn rat was very highly significantly greater than that in fetuses both per wet weight and per tissue nitrogen content.

In the intestines the concentrations were consistently very low, at the lower limit of the method. No significant difference from the fetal values was established as regards tissue nitrogen. As regards wet weight values fetuses under 4 g in weight showed a very highly significantly smaller amount of arylsulphatase; the difference from the larger fetuses was not significant.

(b) Rats aged 1 month. The arylsulphatase concentrations established in 48 rats were 70.3 ± 3.8 U_w and 24.9 ± 1.58 U_N in the liver, 73.1 ± 8.1 U_w and 22.0 ± 0.69 U_N in the kidney and 7.2 ± 0.71 U_w and 2.4 ± 0.22 U_N in the intestine. Compared with newborn rats the increase in activity was statistically very highly significant in the liver by both methods of calculation. The same was true of the kidney in regard to wet weight but in terms of tissue nitrogen the difference was only significant. A significant decrease occurred in the intestinal concentrations in regard to tissue nitrogen; as regards wet weight, the difference was not significant.

(c) Adult rats aged 3 ½ months. The enzyme activity in 54 rats of this group was 109 ± 4.4 U_w and 38.3 ± 1.83 U_N in the liver, 81.0 ± 2.56 U_w and 28.8 ± 1.41 U_N in the kidney and 6.5 ± 0.65 U_w and 3.3 ± 0.56 U_N in the intestine. The increase in the liver was statistically very highly significant both per wet weight and per tissue

Table XIV Rat arylsulphatase concentrations by sexes $\overline{x} \pm s \{\overline{x}\}$ (n)

	(U_w)	(U_N)
liver of rat aged 1 month,		
female	66±5 3 (16)	22 9±2 6 (14)
male	74±5 4 (20)	26 1±2 5 (18)
liver of rat aged 3 ½ months		
female	99±6 3 (26)	38 7±3 0 (19)
male	116±5 4 (30)	38 1±2 6 (23)
kidney of rat aged 1 month		
female	58±5 3 (16)	21 5±1 2 (15)
male	58±4 6 (19)	20 8±1 35 (14)
kidney of rat aged 3 ½ months,		
female	87±3 0 (28)	32 2±2 3 (19)
male	75±4 0 (27)	27 4±1 61 (23)

nitrogen compared with rats aged 1 month The increase was significant in the kidney per wet weight and very highly significant per tissue nitrogen There were no significant changes in the intestinal concentrations

Sex difference

The mean enzyme concentrations in rats of either sex aged 1 and 3 ½ months are given in Table XIV for the liver and kidneys

The sex difference was not statistically significant in either organ in this strain of rat at the age of 1 month Adult rats, on the other hand, according to the wet weight values, showed a statistically significantly greater amount of enzyme in the liver and a significantly smaller amount in the kidney of male No such difference could be demonstrated in the results per tissue nitrogen

B Hydrolysis of steroid sulphates

Hydrolysis in growing rat organism

The means and standard errors for the different age groups are summarised in Table XV It shows the splitting of both DHAS and OS measured in terms of wet weight and tissue nitrogen and the number of analyses made The changes in enzyme activity are also presented graphically in Fig 17

Table XV. Splitting of DHAS and OS by rats of different ages

	DHAS		OS	
	(U_w)	(U_N)	(U_w)	(U_N)
fetus ..	1 1±1 23 (23)	—	8±25 (23)	—
newborn	1 4±1 24 (17)	—	230±47 (16)	69±16 (16)
1 month	29 3±1 62 (28)	10 8±1 10 (28)	440±40 (27)	155±16 (27)
3 ½ months	36 4±1 33 (30)	13.3±1 00 (30)	990±55 (28)	360±21 (28)

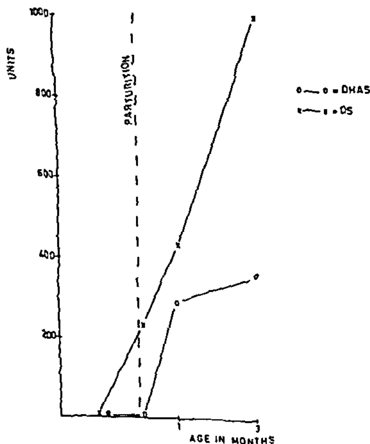


Fig 17 Splitting of DHAS and OS in the liver of growing rat, 0 2 mM steroid sulphate, pH 7 8 in TRIS buffer Results for DHAS multiplied with 10

(a) *Fetuses* The livers of 29 fetuses of different ages were analysed for DHAS giving 1 1 ± 1 23 U_v as *the mean steroid sulphatase* activity This was not significantly different from 0 and the values for activity were both positive and negative Nor was the mean (8 ± 25) for OS significantly different from negative the results were both under and above 0 Steroid sulphates were not hydrolysed by the liver of rat fetus in these experimental conditions

(b) *Newborn* The DHAS splitting ability of the liver of 16 newborn rats was 1 4 ± 1 24 U *This was not significantly different from 0* both positive and negative values were obtained

Only positive values were obtained for the hydrolysis of OS The mean enzyme activity was 230 ± 47 U and 89 ± 16 U_N The rat of newborn age was capable of splitting estrone sulphate but not dehydroepiandrosterone sulphate in these conditions

(c) *Rats aged 1 month* Of the 28 rats studied 14 were male and 14 female *The specific steroid sulphatase content in rats of this age* was 29 3 ± 1 62 U_w and 10 8 ± 1 10 U_N The amount of estrone sulphate hydrolysed was 440 ± 40 U and 155 ± 16 U_N Steroid sulphatase was thus demonstrable no negative values were established The increase in the ability to split estrone sulphate was statistically highly significant in terms of both wet weight and nitrogen compared with newborn rats

(d) *Adult rats aged 3 ½ months* Steroid sulphatase *analysis of the* livers of 30 rats 15 male and 15 female gave in enzyme concentration for the group of 36 4 ± 1 33 U and 13 3 ± 1 00 U_N Activity was a little higher than in rats aged 1 month The difference was not statistically significant The means were 990 ± 55 U and 359 ± 21 U_N for OS In adult rats the splitting activity of the liver for OS was very highly significantly greater than in rats aged 1 month

(e) *Sex difference* The steroid sulphatase activity of male rats aged 1 month was 31 4 ± 4 5 U and of female rats 27 8 ± 4 5 U_N The difference was not significant Nor was the difference significant per tissue nitrogen content (10 8 ± 1 52 and 10 8 ± 1 61 U_N) For adult male and female rats the corresponding enzyme activity means were 36 3 ± 4 13 and 37 3 ± 3 81 U and 13 1 ± 1 55 and 13 5 ± 1 23 U_N The differences between the sexes were not significant

The OS splitting activity in male and female rats aged 1 month was 440 ± 56 and 390 ± 64 U and 162 ± 26 and 150 ± 20 U_N The differences were not statistically significant The corresponding values for

adult rats were 950 ± 24 and 1020 ± 33 U_w, and 360 ± 29 and 355 ± 33 U_λ. The differences were not significant. It was thus impossible with the methods and rat strain used to establish sex differences in the hydrolysis of steroid sulphates.

Human placenta as a splitter of steroid sulphates

Specific steroid sulphatase was found to be very profuse in the human placenta. The mean amount was 173 ± 20 U_w and 75 ± 7.4 U_λ on the maternal side and 187 ± 20 U_w and 71 ± 4.5 U_λ on the fetal side. The amount was larger than e.g. the rat liver concentrations ($p < 0.01$). There was no significant difference in localisation between the two sides.

Estrone sulphate was also split vigorously by placenta homogenates. The unit quantities obtained were 860 ± 71 U_w and 345 ± 32 U_λ on the maternal side and 885 ± 80 U_w and 350 ± 34 U_λ on the fetal side. The means were roughly the same on both sides and the difference was not statistically significant.

Human fetus and steroid conjugates and the hydrolysis of androsterone sulphate

The liver of all the 6 fetuses studied was capable of splitting estrone sulphate vigorously. One per cent homogenate split 50—80 per cent of the substrate during an incubation of 20 hours. No hydrolysis of steroid sulphates was observed in these few experiments in the kidney, adrenal gland, pancreas and spleen. DHAS was not split by any tissue in human fetus. Like the placenta of fullterm babies the fetal placenta was able to split steroid sulphates in the 3 cases studied.

Human alimentary canal samples obtained at operations, various tissues of the human fetus, tissues of rats of various ages and human placentas were used as the source of the enzyme to study the hydrolysis of androsterone sulphate. Potential splitting was studied at different pH (5—9) levels by incubating AS with 10 per cent homogenate for 6—20 hours. No hydrolysis of AS was observed in any of the samples.

IV

DISCUSSION

There can be some uncertainty about using whole homogenate as the source for studying an enzyme Several fractions can sometimes be described by the same enzyme name, and when this happens it is not known what is being measured Against this however, it is arguable that it has not been possible to isolate arylsulphatase in perfectly pure different fractions NPS, used in the present work, is known as the substrate of fraction C which it splits However, fraction A also has some affinity towards NPS (DODGSON, SPENCER and WYNN 1956) Fraction B does not split NPS at all The optimum reaction milieus of different fractions for the same substrate vary The optima obtained and used in the present work for human fetus were identical with those obtained by DODGSON, SPENCER and WYNN with pure fraction C (1956), for liver optimum pH 7 3 and optimum substrate concentration 0 003 M The splitting ratio of NPS and nitrocatechol sulphate (corresponding approximately to fraction C and fraction A + B) in man is 1 04 in liver, 1 05 in pancreas 1 16 in kidney 1 34 in lung 1 41 in brain, 1 22 in heart 1 23 in large intestine and 1 17 in small intestine It is possible that owing to its obvious more profuse presence especially in lungs and brains but also in kidney and intestine, there may have been a small amount of fraction A in the analysis It can be concluded that in the present work C was at least the predominant fraction, if not the sole fraction determined from the human fetus

The optimum results for rat tissues are also interesting DODGSON, SPENCER and THOMAS (1955) obtained 7 0 as the optimum pH for fraction C and 5 8 for fraction A The attention is drawn by the fact that different optima for two different tissues liver and kidney, 7 0 for the former and 5 8 for the latter were obtained in the present work There might thus also be great differences in the localisation of the fractions in the various organs as has earlier been shown to be the case in part in human tissues

Table XVI Arylsulphatase of acetone dried adult tissues according to Dodgson, Spencer and Wynn (1956) compared with that of fetal crude homogenates in fairly similar incubation conditions in the present work Results as calculated as µg liberated p-nitrophenol / gr wet wt / hr

Tissue	Dodgson	Present author
liver	35500	12580
pancreas	10600	1800
kidney	13700	1240
spleen	3500	45
small intestine	2500	5970 (jejunum)
lung	1150	490
large intestine	950	4240
brain	510	135
heart	100	70

The use of fresh rat liver homogenate has been warned against, not only for the lack of a more accurate definition of the fraction but also because of the metabolisation of p nitrophenol Obviously, however, this drawback did not affect the present work The rat strain may be to blame for the failure to metabolise p-nitrophenol Reference may be made here to earlier reports that major metabolisation was not observed in the other tissues in either mice or rats (DODGSON and SPENCER 1953a)

There are no reports of systematic observations of the stability of sulphatase It generally remains stable for several months at —15°C The present results confirmed that it keeps relatively well at —15°C, for months even Assessed against the heat inactivation of enzymes in general it can perhaps be said that sulphatases rank somewhere in the middle as regards stability

The localisation of arylsulphatase in the different organs in human fetus revealed a fair similarity with the findings of DODGSON SPENCER and WYNN (1956) in their study of enzyme activity in adult man For comparison of the activity table XVI shows the results of their study and the present results, both calculated as µg of liberated p nitrophenol per g of wet weight tissue, the pH and the substrate concentration were the same in practice

The results are naturally not fully comparable There is no doubt that DODGSON SPENCER and WYNN measured fraction C in their investigation, while there was no full certainty of the fractions in the present work The substrate was the same, also the buffer and its pH Another differ-

ence was the age of the material DODGSON SPENCER and WYNN used material from adults who had been dead for some time the present author used fresh fetuses

It is to be noted *however that in both adults and fetuses liver is the* organ that contains the most arylsulphatase DODGSON found profuse enzyme in the pancreas and the kidney and the same observation was made in the present investigation However with this method the activity in these fetal tissues was only 1/10 of its level in an adult while in the liver it was *1/3* of the latter The comparison is not really valid of course since among other things the homogenate percentage (0 2) used in the present work was fairly low for liver and the homogenate percentage for pancreas and kidney was 1 In fetal intestine however arylsulphatase was most profuse in the region of the small intestine as was the case in adults Taken as a whole the intestine is much richer in enzyme in the fetus than after the termination of extrauterine *life This may be attributed in part to meconium* one important component of which derives from arylsulphatase rich liver in part to the paucity of enzyme poor connective tissue Arylsulphatase is in fact localised in the intestines in the mucosa (PULKKINEN unpublished observation) as it usually is in the epithelial cells (RUTENBURG *COHEN and SELIGMAN 1952) On the other hand when* all the meconium had been washed away carefully with water no great fall in enzyme activity ensued in the present work suggesting that the fetal intestine unlike the adult intestine is rich in arylsulphatase richer than the pancreas or the kidney

The greatest difference seen in the comparative *table however is in* the values for the spleen The values for human fetus are at the lower limit of the method while values have been recorded for adults which are c 1/4 of the activity of the kidney and in the size class of the intestines It might be thought that there is an inhibitory factor in the fetal spleen but incubation of splenic homogenate *with hepatic homog*enate produced no fall in the activity of the latter On the other hand the function of the spleen in the fetal period as a blood forming organ is known to differ from that in the adult The difference observed might be a manifestation of this

The lung brain and heart are otherwise engaged in *both the investi*gations in the same order of activity The concentration in the lung in *both investigations* was 2—3 times greater than in the brain The cardiac muscle was the organ poorest in arylsulphatase

Viewing the results against those arrived at by HUGGINS and SMITH (1947), no quantitative comparison proper is possible as these workers used a pH and substrate concentration considerably lower than the optimum They also observed that activity in the adult was most profuse in the liver The spleen, too was also fairly rich in arylsulphatase Worthy of special mention is the fact that they studied at the same time the cardiac and skeletal muscle and found the former to have more arylsulphatase, as in the present work

The literature also has a mention of an attempt to determine the arylsulphatase of bone marrow (FOLLS 1951) Hardly any activity was observed and none at all was demonstrated in the cartilage The same observation applied to the fetus

The main period of fetal development in question in the present investigation was the organogenetic period In all the tissues with activity of measurable concentration the tendency was a rising one with the development of the fetus and this applied to both wet weight and tissue nitrogen values The failure to establish a statistically significant increase in only two tissues may have been due to the shortness of the period of observation for human fetuses This arose from the natural limitations governing the performance of *sectio minor*

A very pronounced regression was established between fetal length and arylsulphatase concentration in the kidney and the lung It is difficult to say why this is so just in these tissues Considered methodologically these two organs contain a larger amount of fraction A than the liver The lung is embryologically tissue which must develop to full functional readiness before birth but which does not function during fetal life Nearly the same can be said of the kidney for metabolism does in fact take place via the placenta Metanephrogenic tissue diminishes throughout fetal development and nephrons are formed all the time (STARCK 1955) The difference e g from the development of the liver is distinct in this respect since for reasons of metabolism the liver must in many respects be capable of function in the fetal period already

The arylsulphatase activity of the kidney and the lungs rises almost equally sharply and relatively linearly, and the shape of the curve does not help to illustrate the point any better As arylsulphatase in the kidney has been localised in the nephrons (RUTENBURG COHEN and SELIGMAN 1952) linear increase concurs with the embrylogical finding that the nephrons likewise increase relatively evenly

Compared with other enzymes, the results obtained for the change in arylsulphatase concentration are not always analogous. SAVOLA (1957) studied the β glucuronidase content of human fetuses and found that it rose fairly sharply also in liver. There are in fact several reports in the literature that β glucuronidase and arylsulphatase do not run a parallel course (DODGSON, LEWIS and SPENCER 1953, HAYASHI et al 1955a and b, HAYASHI et al 1957, SELIGMAN, NACHLAS and COHEN 1950).

The arylsulphatase of the placenta has been studied earlier (BIANCHI 1955a, BIANCHI and VALLI 1955). Their findings for the quantity of the enzyme are 1/10 of the values obtained in the present study, the reaction milieus were different. BIANCHI washed the placentas with physiological saline (activator) but made his measurements at a more acid pH (inactivation). He attached great importance to arylsulphatase in placenta and said that arylsulphatase causes changes in the metabolism of hypertensive amines because the arylsulphatase content is lower in toxemia. The pre-eclamptic placentas included in the present series do not support this view but the materials of both investigations were too small for proper evaluation of the question.

The water content of the earlier fetal period is great. This makes it understandable that a significant regression was established between fetal length and enzyme activity of corresponding placenta in the wet weight analysis. No increase occurred in the results per tissue nitrogen during the observation period. Clearly, the intrapartum placenta possesses a considerably higher arylsulphatase content per weight unit although not per unit of tissue nitrogen. Without forgetting the role played by arylsulphatase in the metabolism of phenol sulphates mention might be made in this connection of the free estrogens circulating in the maternal blood at birth (REYNOLDS 1949). For example, estrone sulphate is split into free estrogen by arylsulphatase. On the other hand the clearance of sulphates in the kidney is smaller than that of glucuronates (KELLIC and SMITH 1957). The sulphate form is probably more common in the blood, glucuronate is more a secretory form. The placenta (also the fetus itself) possesses higher arylsulphatase concentrations during birth, i.e. a greater capacity to split estrogen sulphates. This is surely not the sole explanation of the phenomenon but it may be a part factor. In addition to the rise in the splitting capacity in the placenta per gramme the total weight also rises.

Following the arylsulphatase concentrations in rat material during

intrauterine life gives a clear idea of the real increase in the enzyme towards birth. The phenomenon can be regarded as one of many that prepare the organism for the more mature extrauterine life. Many of the natural substrates possessed by arylsulphatase become important for the individual once extrauterine life has started, the time when the biological factor studied here is generally ripe to assume its tasks.

The most distinctive detail in the organ distribution of arylsulphatase in human and rat fetus is the almost complete absence of the enzyme in the intestines of the rat.

No statistically significant increase with age was established in human fetal liver during the observation period but there was a rising tendency. Rat fetuses displayed a distinct rise (r = 0.525 and 0.392 for the U_w and U_N values). It is possible that a corresponding development occurs during the intrauterine evolution of man although it was not observed in the present work owing to natural limitations.

In kidney, again, a very highly significant increase in arylsulphatase was established in both parts of the material. The relatively very close r values (for human fetus r was 0.713 and for rat fetus 0.619 in the wet weight analysis) and the sharpness of the rise seem to be a general feature. The purpose for which the kidney secreting arylsulphates via glomerular filtration (KELLIC and SMITH 1957) needs the arylsulphatase rests on mere speculation. The function theory introduced by SUZUKI, TAKAHASHI and EGAMI (1957) may be mentioned again in this connection. As arylsulphatase is able to transfer the sulphate from an arylsulphate to another aryl group, the kidney may be able to affect this secretion and make it selective.

Comparison of the arylsulphatase concentration of newborn rat with that of large fetuses close to birth gave values of the same magnitude. Hence no major changes in the enzyme content occur around birth; the development that has begun goes on fairly evenly. The change to extrauterine life has no especial influence on the content of the enzyme.

During the development of rat to sexual maturity the enzyme concentrations increase in both liver and kidney. It is obvious that age affects the concentrations later, too, and that the level does not remain stable throughout life. The maximum age (c. 30 years) of the arylsulphatase of human urine established by AMMON and NEY (1957), for example, speaks in favour of this assumption.

A sex difference was established in the enzyme of rats (DODGSON, SPENCER and THOMAS 1953b). It was demonstrated in rats of the Medical

Compared with other enzymes, the results obtained for the change in arylsulphatase concentration are not always analogous. SAVOLA (1957) studied the *β-glucuronidase content of human fetuses and found that it rose fairly sharply also in liver.* There are in fact several reports in the literature that β-glucuronidase and arylsulphatase do not run a parallel course (DODGSON, LEWIS and SPENCER 1953, HAYASHI et al 1955a and b, HAYASHI et al 1957, SELIGMAN, NACHLAS and COHEN 1950).

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Following the arylsulphatase concentrations in rat material during

vation for the steroid balance during pregnancy. Free steroids in general are regarded as having a stronger influence than conjugated steroids (BISHOP, RICHARDS and PERRY 1951). Many estrogen sulphates are substrates of unspecific arylsulphatase and the fetus thus 'produces' free estrogens. The fetus does not interfere with the 5 α- and Δ^5-steroids of 3 β sulphates since there is no specific steroid sulphatase. The free steroid form of DHAS used and common in nature, i.e. dehydroepiandrosterone, is probably of no great importance for the pregnant organism.

Specific steroid sulphatase also appears soon after birth. The quantity found in a rat of no more than 1 month of age is almost the same as in an adult animal. There is no absolute certainty whether it increases further from this level towards adulthood. At least the tendency could be seen. A part of the natural substrates of specific steroid sulphatase are sex hormones, but there are also fairly numerous conjugated secreta of the adrenal gland. It is thus understandable that a month-old rat can have such a marked steroid sulphatase content in liver.

No sex differences were established for this enzyme. The explanation again may be the strain used; the limitations of the method must also be considered.

The splitting of estrone sulphate increased statistically significantly throughout the period studied. The result is analogous with the finding for NPS. With the exception of substrates for arylsulphatase in estrone sulphate this can be regarded as natural. Estrogen production increases and at the same time there is an increase in the enzyme which regulates the balance between free and conjugated hormones and thus, via their physiological effect of different value, the changes in the organism displayed by these hormones.

The relatively high concentrations of steroid sulphatase in the placenta cannot be without biological significance. The specificity of the enzyme makes it interesting in this respect. The purpose probably is to be able to liberate during pregnancy an increasing number of steroid conjugates as free hormones. The presence in the placenta of an enzyme with many kinds of substrates (such as arylsulphatase) cannot be considered binding proof of its effect on steroid metabolism. But as far as steroid sulphatase is concerned there is no room for interpretation in this sense — it affects steroid sulphates only. In addition, some other steroid sulphates are also readily hydrolysed, e.g. the estrone sulphate used here.

Research Council The analysis was confined to the wet weight of liver enzyme with p acetylphenyl sulphate as the substrate The present investigation established a corresponding significant difference in adults though relatively it was much smaller This difference may be due e g to the rat strain used In the kidney the difference is the reverse Such a phenomenon is not unknown enzymologically For instance, the β glucuronidase concentration in the kidney increases when androgen is used, *but this does not occur in the liver* (Fishman and Farmelant 1953) It has been demonstrated that œstrone, on the other hand, increases the liver β glucuronidase of ovariectomised mouse (Kerr Campbell and Levvy 1949) As is only natural, no sex difference was established in this study in the sexually immature rats of the strain used (i e rats aged 1 month) The difference emerged only with sexual maturity However, the phenomenon was not observed in the nitrogen values and it is therefore possible to speak only of a certain tendency

The β glucuronidase concentration of rat liver and serum is increased in pregnancy (Bernard and Odell 1950) A direct consequence of this is that conjugates can be freed most effectively during pregnancy *The ratio between 17 hydroxyketosteroids and its conjugates is changed* during pregnancy, free steroids become relatively much more numerous (Pekkarinen and Rauramo 1960) In addition to lowered conjugation capacity, this may be due also to the possibility that conjugates are hydrolysed more rapidly than usual in this condition A source of great potential increase in this respect is the ability of the placenta to split steroid conjugates extremely efficiently

In addition to the pronounced arylsulphatase activity established and its increase with the maturing of the placenta, the placenta also contains profuse β glucuronidase (Fishman 1955) which splits the corresponding conjugates of glucuronic acid

The absence of specific steroid sulphatase in the fœtus and the new born can be regarded as an indication of their insufficient steroid metabolism It was seen that rat fetuses did not hydrolyse estrone sul phate although they possessed arylsulphatase and this sulphate should be split by the same enzyme *The method used* however, makes it uncertain which fraction is being measured with the crude homogenate Secondly, there are no detailed studies to clarify which fraction splits estrone sulphate The matter calls for further investigations It has been demonstrated, nevertheless, that human fetal liver *splits estrone sul* phate as expected It is difficult to assess the importance of this obser

sulphate and 3α steroids as glucuronate The adrenogenital syndrome of puberty which also displayed adrenal hyperplasia was an exception In this fairly abundant androsterone sulphate was excreted which was not normally observable even when the patient was given 100 mg of androsterone per os In conditions like these where androsterone sulphate synthesis is possible its cleavage might also be established with biological material which cannot be done in a normal organism

The natural function of sulphatases is to split conjugates in vivo Their activity results first in fewer conjugates and secondly in more numerous liberated phenols steroids etc As regards the first alternative relatively little is known of the biological tasks of sulphate conjugates If the other component is the poisonous phenol it must be merely a kind of excretion that is involved a mode of detoxication An example of the causal relationship that may be involved is the correlation between certain carcinogenic amines their sulphate conjugates and sulphatase in patients with cancer of urinary bladder The normal metabolites of tryptophan i e 3 hydroxyanthranilic acid and 3 hydroxykynurenine are carcinogenic when implanted in mouse bladder (BONSER et al 1953) They occur chiefly as conjugates in the urine of healthy persons (BOYLAND and WILLIAMS 1955) whereas patients with cancer of the urinary bladder have a relatively greater number of free carcinogens It is known that carcinogens are generally conjugated e g into sulphates in which form they lose their carcinogenicity Moreover sulphatases is generally unable to split these conjugates (BOYLAND et al 1956) It has been established however that the urine of patients with cancer of the urinary bladder contains an abnormally high amount of sulphatase (BOYLAND WALLACE and WILLIAMS 1954 DZIALOSZYNSKI 1957) and consequently there may be a causal relationship

The old argument that sulphate conjugates are more inactive than free sulphates is only partly true The contrary has been suggested and supported by evidence For instance the sulphate conjugates of diethylstilbestrol estradiol and estrone inhibit kynurenine transaminase *in very low* concentrations while free estrogens are ineffective even in saturated solutions (MASON and GULLEKSON 1960) Hence if sulphatase is used to split steroid conjugates *in vivo* stimulation may occur in some function but in the light of the example above it is also possible that retardation may occur

It is difficult to assess the *in vivo* effect from *in vitro* experiments

6

To return to 3 α steroid sulphates, it must be remembered first that the form is not physiological (ROY 1956c), these steroids tend to become glucuronates, the form in which they are secreted (STAIB, TELLER and SCHARF 1960) Androsterone sulphate did not in fact split the tissues of any species in the different experimental conditions This may also be regarded as proof of the biological task of the sulphatase which really split conjugates and which have their own substrates in nature The placenta, no more than any other of the tissues studied, was unable to produce androsterone in free form

No differences were observed in the enzyme concentrations of the different sides of the placenta Macroscopic cutting probably gives fairly similar tissue On the other hand, the placenta is composed almost entirely of fetal material (STARCK 1955)

The literature unfortunately contributes nothing to the histochemical localisation of sulphatases in the placenta It is generally assumed that steroids are localised in syncytiotrophoblasts (WISLOCKI and BENNETT 1943)

It is noteworthy that the placenta of fetuses contains less arylsulphatase A notable histological feature is the disappearance of Langhans' cells by the 4th—5th month of pregnancy (STARCK 1955) However, as the enzyme concentrations are higher in intrapartum placenta than in the 2nd—5th month of pregnancy it might be assumed that, like the steroids, sulphatases are localised in syncytiotrophoblasts

Does steroid sulphate split *in vivo*? It has been observed that if a female rat is given ^{35}S sodium estrone sulphate intravenously, subcutaneous or by tube into the stomach, 75 per cent of the radioactive ^{35}S estrogen is found within 36 hours in the urine and only in free form (HANAHAN and EVERETT 1950) Fifteen per cent is excreted into the bile ^{35}S sodium estrone sulphate administered to a pregnant rat did not split when it penetrated the placenta on the fetal side (HANAHAN, EVERETT and DAVIS 1949) The result is fully analogous with that obtained in the present investigation with the *in vitro* analysis of liver homogenates of rat fetus It should be pointed out in this connection that human fetal liver was capable of hydrolysing estrone sulphate The missing splitting capacity of the substrate was thus obviously specific for the strain

The synthesis has been known long *in vivo* Among the most recent studies is that of STAIB, TELLER and SCHARF, 1960, who established that 3β steroids not unlike dehydroepiandrosterone were excreted as

V

SUMMARY

(1) Arylsulphatase was determined by modification of the p-nitrophenol sulphate method and the splitting of steroid sulphates was studied according to Roy by spectrophotometric measurement of the unsplit steroid sulphate as methylene blue salt extracted with chloroform.

The pH optimum of arylsulphatase was 7.3 in man, 7.0 in rat liver and 5.8 in rat kidney. The optimum substrate concentration in human fetus was 0.008 M for p-nitrophenyl sulphate. No metabolisation of p-nitrophenol was established in vitro in these experimental conditions in rat or human fetal tissues. The recovery of protein-containing material was good when zinc hydroxide was used for precipitation. The maximum absorption of p-nitrophenol was at 402 mμ. The enzymatic reaction was relatively linear in the period studied.

Arylsulphatase kept well for one month at $-15°$ C. At $+4°$ C there was a relatively rapid decrease in enzyme activity and after 24 hours at $+20°$ C only a part of the activity remained.

(2) Dehydroepiandrosterone sulphate, estrone sulphate and androsterone sulphate were employed as steroid sulphates. The optimum pH of human placenta and rat liver was approximately 7.8 in TRIS buffer when the substrate concentration was 0.2 mM.

The absorption maximum of the complex of steroid sulphate methylene blue extracted with chloroform was 663 mμ in an alcoholic solution. The hydrolysis was relatively linear to the reaction time with an incubation of 3—6 hours. The maximum substrate hydrolysing degree was 50 per cent.

(3) The human fetus series was collected from sectio minor operations for legal abortions. It consisted of 73 fetuses with a crown heel length of 0.5—33 cm. A total of 811 duplicate determinations of arylsulphatase was made on this part of the material.

The amount of phosphate contained in the kidney physiologically, e g in intracellular fluid, is capable of considerable inhibition of aryl sulphatase A and B

These few aspects perhaps illustrate the rôle of sulphatase The results obtained in the present work support the view that the function of sulphatases must be comparatively wide The localisation in so many organs, its increase as the organism grows and the high concentrations in the steroid producing placenta are such facts

old) rats were analysed for arylsulphatase Intestinal tissue gave small enzyme activity values The hepatic arylsulphatase concentration grew from 26 1 ± 1 75 U_w at birth to 109 ± 4 4 U_w in adults and 11 9 ± 0 87 U_N to 38 3 ± 1.83 U_N The increase was significant also between newborn and month-old animals The corresponding values for the kidney were 32 5 ± 2 2 and 81 0 ± 2 56 U_w, 17 7 ± 1 93 and 28 8 ± 1 41 U_N

No definite sex difference was established in the rat strain used The mean for male rats was greater than the mean for the females, and in the liver the difference was statistically significant per wet weight The kidney gave the opposite result

(6) Six human fetuses and 103 rats were used to study the hydrolysis of steroid sulphates The tissues did not hydrolyse androsterone sulphate even when the reaction conditions were modified

Steroid sulphatase was not observed in the eigth tissues of human fetus studied, nor in the liver of rat fetus or newborn rat The liver of month-old rat, on the other hand, has almost the same level of enzyme activity as an adult rat No sex difference was observed in the rat strain used

Estrone sulphate was not hydrolysed in rat fetal liver, but the liver of human fetus was able to split it easily Newborn rat possesses the splitting ability, a capacity which grows in the animal as it develops

(7) Twenty nine placentas from spontaneous deliveries and six fetal placentas were studied for their ability to hydrolyse steroid sulphates Human placenta in all its phases contains profuse steroid sulphatase and splits estrone sulphate readily No significant difference was established between the samples from the fetal and those from the the maternal side

Relatively high arylsulphatase activity was established in some tissues of the human fetus The results were expressed per μg of liberated p nitrophenol/100 mg wet wt /hr (U_w) and per mgN/hr (U_N) The liver showed the highest enzyme concentration (1260 ± 70 U_w and 770 ± 50 U_N), followed by the ileum (675 ± 50 U_w and 410 ± 36 U_N), *jejunum, duodenum, colon, pancreas (186 ± 17 U_w and 134 ± 12 1 U_N)*, kidney (124 ± 8 8 U_w and 118 ± 8 1 U_N), lungs (40 3 ± 3 6 U_w and and 47 ± 3 7 U_N), brain (13 5 ± 1 24 U_w and 17 7 ± 1 48 U_N), genitals, salivary glands and adrenal gland The amounts in the heart, thyroid gland, spleen and skeletal muscle were at the lower limits of the method and no activity at all was established in bone marrow and bone

A statistically significant increase in arylsulphatase activity was established in human kidney and lung during the period of fetal growth observed

(4) Arylsulphatase activity was established in 55 fetal placentas *It was 191 ± 11 2 U_w* and *183 ± 14 1 U_N* in the samples taken from the fetal side and 171 ± 12 1 U_w and 156 ± 15 6 U_N in those taken from the maternal side There was no significant difference between the two sides

In the 29 placentas from spontaneous deliveries the activity was 218 ± 17 U_w and 86 6 ± 6 90 U_N in the samples from the fetal side and 241 ± 15 1 U_w and 104 ± 5 2 U_N in those from the maternal side The placenta showed a significantly greater amount of arylsulphatase *per weight unit during delivery than in the fetal period* The reverse was true when measured in terms of tissue nitrogen

(5) In rat arylsulphatase activity occurs at least in the liver and kidney during intrauterine development *Analysis of the tissues of* 65 fetuses revealed a significant increase in the activity of both these organs with fetal growth, and this is true even for the results calculated per tissue nitrogen which excluded the water content The mean concentration in the liver in fetuses of different ages was 16 0 ± 1 12 U_w and 9 7 ± 0 27 U_N and in the kidney 12 9 ± 0 97 U_w and 7 6 ± 1 11 U_N

The alimentary canal of rat fetus contained very little arylsulphatase, 6 3 ± 0 31 U_w and 4 6 ± 0 25 U_N, the difference from the human fetus was distinct

The increase in activity continued post partum The liver kidney and *intestine of 46 newborn, 48 month old and 54 adult* (3[1]/[2] months

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OSSED SPINAL REFLEX ACTIONS OKED BY VOLLEYS IN SOMATIC AFFERENTS

BY

BIRGITTA HOLMQVIST

LUND 1961

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Introduction

SHERRINGTON and his co workers described and analysed a number of ipsilateral and crossed reflexes in decerebrate and spinal cats (and dogs) Subsequent electrophysiological work mainly on ipsilateral reflexes has aimed at disclosing the contributions from specified afferent systems and at revealing the neuronal paths for the effects

The crossed reflexes to muscles can be classified in two main groups One category are those evoked by limb movements and the other are those appearing with the ipsilateral flexion reflex In the former group a number of reflexes have been described The best known of these reflexes Philippson s reflex is a contraction of the contralateral knee extensors evoked by forced flexion at the opposite knee (PHILIPPSON 1905 SHERRINGTON 1908 1909 a) Philippson s reflex is the crossed correlate to the ipsilateral lengthening reaction Another crossed reflex with relaxation of knee extensors and contraction of knee flexors has been correlated with the ipsilateral shortening reaction (SHERRINGTON 1908 1909 a) A detailed description of crossed reflexes evoked by flexion or extension of the hip or ankle has also been given by SHERRINGTON (1908 1909 a)

Of the crossed reflexes appearing with the ipsilateral flexion reflex the most frequent reaction is contraction of hip knee and ankle extensors and relaxation of the antagonist flexors (SHERRINGTON 1906 1910) More seldom there is crossed flexion with contraction of flexors and relaxation of extensors (GRAHAM BROWN 1911 1912 *1914* GRAHAM BROWN and SHERRINGTON 1912) Characteristic differences between the ipsilateral flexion reflex and the crossed extension reflex were described by LIDDELL and SHERRINGTON (1923 a b cf CREED DENNY BROWN ECCLES LIDDELL and SHERRINGTON 1932)

It is now known that reflexes can be mediated by two routes either directly to alpha motoneurones or indirectly via the gamma loop (cf GRANIT 1955) HUNT (1951) has found that crossed actions are exerted on gamma motoneurones Hence crossed reflexes may be evoked via the gamma loop but the crossed reflexes discussed above were also found after interruption of the gamma loop by de afferentation on the testing side

LUND 1961
CARL BLOMS BOKTRYCKERI A B

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Of the crossed reflexes appearing with the ipsilateral flexion reflex the most frequent reaction is contraction of hip knee and ankle extensors and relaxation of the antagonist flexors (SHERRINGTON 1906 1910) More seldom there is crossed flexion with contraction of flexors and relaxation of extensors (GRAHAM BROWN 1911 1912 1914 GRAHAM BROWN and SHERRINGTON 1912) Characteristic differences between the ipsilateral flexion reflex and the crossed extension reflex were described by LIDDELL and SHERRINGTON (1923 a b cf CREED DENNY BROWN ECCLES LIDDELL and SHERRINGTON 1932)

It is now known that reflexes can be mediated by two routes either directly to alpha motoneurones or indirectly via the gamma loop (cf GRANIT 1955) HUNT (1951) has found that crossed actions are exerted on gamma motoneurones Hence crossed reflexes may be evoked via the gamma loop but the crossed reflexes discussed above were also found after interruption of the gamma loop by de afferentation on the testing side

As described above SHERRINGTON correlated the crossed reflexes evoked from muscles with the ipsilateral reflexes It is now assumed that the ipsilateral shortening reaction is caused by impulses in I a afferents (with *annulo spiral endings on muscle spindles)* and the lengthening reaction by impulses in I b afferents (from Golgi tendon organs) (cf GRANIT 1955 ECCLES 1957, LUNDBERG 1959, LLOYD 1960, HUNT and PERL 1960) If the crossed correlates of these reflexes were evoked from the same afferent systems their reflex actions would be in agreement with the scheme of double reciprocal organization In a recent electrophysiological investigation PERL (1958) examined the crossed effect of volleys in group I muscle afferents and has suggested that the crossed actions from the I a and I b systems of knee extensors and flexors are organized in a double reciprocal fashion Support for this view was reached in experiments with adequate stimulation of muscles (PERL 1959)

The aim of the present work was to investigate further the actions by volleys in somatic myelinated afferents on contralateral alpha moto neurones and the supraspinal control of these crossed paths Preliminary reports of the results have been given (HOLMQVIST 1960, 1961)

Methods

The experiments were performed on unanaesthetized cats. During the operation ether mixed with suitable amounts of O_2 and air was given but at least 2—3 hours elapsed between the withdrawal of anaesthesia and the beginning of the experiment. In some experiments the blood pressure was continuously measured. If there were signs of vascular failure a solution of Dextran was given intravenously. The cats were paralyzed with Flaxedil and artificially respirated.

Two main types of preparations were used. One type was decerebrated by intercollicular section. In these experiments the cerebellum was sometimes removed by suction in order to expose the brain stem to permit subsequent lesions in the lower pons. The lesions were made with a spatula 3 mm wide, as described by HOLMQVIST and LUNDBERG (1961). To obtain a low spinal preparation the cord was transected in Th_{13}—L_1. The other type of preparation was cats made spinal (L_1 or L_2) under aseptical conditions 10—40 days before the final experiment. These cats developed lively spinal reflexes. The classical flexor reflex as well as the crossed extensor reflex could easily be elicited and many cats in addition showed stepping reflexes. A few of these chronic spinal cats were decerebrated in the final experiment but in most of them the carotid arteries (external carotid, lingual, internal carotid, occipital and ascending pharyngeal arteries) were ligated bilaterally in order to deprive the cerebrum of some of its blood supply (VOORHOEVE 1960). These preparations were easier to maintain in a good condition than the decerebrated ones.

The experimental procedure used for investigating crossed reflex actions was similar to that used in investigations on ipsilateral actions (ECCLES and LUNDBERG 1959 a, b). Both hindlimbs were denervated with exception of some hip muscles. The action of conditioning volleys was tested on contralateral monosynaptic reflex discharges recorded from the cut ventral roots. On the conditioning side the ventral roots were usually left intact. In order to obtain a larger and more adjustable monosynaptic reflex, two test volleys were often used. The first volley was subliminal for reflex discharge and preceded the second by an interval giving maximal facilitation of the

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The aim of the present work was to investigate further the actions by volleys in somatic myelinated afferents on contralateral alpha moto neurones and the supraspinal control of these crossed paths *Preliminary* reports of the results have been given (HOLMQVIST 1960 1961)

dispersion across the screen the other not (cf Fig 4) The apparatuses have been constructed by Mr E EIDE

Special care was taken to disclose from which afferent fibre system crossed actions could be evoked Thus series were always taken with fine gradation of conditioning stimulus strength expressed as multiples of the threshold strength for the nerve if not otherwise mentioned When a train of conditioning volleys was given the stimulus strength was kept the same for all impulses The incoming conditioning volleys were recorded in the entry zone of the dorsal roots When the group I actions were investigated discriminative tests were regularly performed to examine the threshold separation between the fast and slow component of group I fibres (BRADLEY and ECCLES 1953 ECCLES ECCLES and LUNDBERG 1957) and always used to decide the maximal group I strength This double volley test was performed in immediate connection to the series investigated

For stimulation condensor discharges (half time decay 45 μsec) were used C-fibres were not activated by these stimuli

The nerves supplying the following muscles were used and the abbreviations given below will stand for them

Q —quadriceps (including all components)
VCr —vastus lateralis vastus medialis and crureus
R =rectus
Gr =gracilis
BSt =posterior biceps and semitendinosus
G —gastrocnemius and soleus
med G=medial gastrocnemius
Pl =plantaris
FDL =flexor digitorum longus and flexor hallucis longus
DP =extensor digitorum longus and tibialis anterior

In addition

Sur =the sural nerve
Joint —the posterior knee joint nerve
FRA =flexor reflex afferents group II and III muscle afferents high threshold joint afferents and skin afferents (ECCLES and LUNDBERG 1959 a)

The abbreviations i and c will be used in the illustrations and stand for *ipsilateral* and *contralateral*

The term *leg* will be used to denote the part of the hindlimb between the knee and ankle joints

monosynaptic reflex evoked by the second volley (ECCLES and LUNDBERG 1959 b). It was always controlled that there remained an adequate subliminal fringe. In a few cats the crossed actions were tested without laminectomy on monosynaptic reflex discharges evoked and recorded in the peripheral muscle nerves. The recording electrodes were placed peripherally to the stimulating ones. To obtain the monosynaptic reflex the stimulus strength was adjusted to activate a maximal amount of Ia fibres without activating efferents antidromically.

Two techniques were employed to record the effect of conditioning volleys. Either the monosynaptic test reflex discharge was recorded at 10—15 fixed intervals after the conditioning volley, or many test reflexes were scattered at all intervals after the conditioning volley and superimposed in one exposure. With the former method interaction curves at several conditioning strengths were obtained by plotting the height of the conditioned monosynaptic reflex expressed in percentage of the height of the unconditioned test reflex with time along the abscissa. Each value was obtained from the mean of 10 superimposed records taken at a frequency of about 1/sec (cf. for example Fig. 1). With the other technique about 100—200 monosynaptic reflex discharges were superimposed in one exposure. Conditioning volleys were given at a fixed interval after the sweep start. At each successive sweep the monosynaptic reflex discharge was automatically delayed so that the test reflex was made to traverse the screen. This procedure was repeated 3—5 times. The CRO spot brightness was kept very weak for the baseline and was increased during the discharges. In parallel on another beam an integrated curve was recorded, which consisted of more or less confluencing spots, each spot recorded simultaneously with a monosynaptic reflex discharge. To obtain this curve the monosynaptic discharge was passed through an integrating network with a time constant chosen to avoid the recording of spontaneous fluctuations of short duration in the height of the monosynaptic discharges. Hence short lasting effects evoked by the conditioning volley are not recorded and these curves can only serve as a complement to the picture given by the monosynaptic discharges. It should also be noted that the time constant of the circuit leads to a displacement in time of the effect on the integrated curve in comparison with the effect on the monosynaptic discharges. The direction of this displacement depends on whether the monosynaptic reflexes have been automatically delayed along the time axis (from left to right on the records, cf. Fig. 6) or in the opposite direction (cf. Fig. 3). Both techniques have been employed as two apparatuses with these different characteristics have been used. Another difference between these apparatuses is the distribution of the discharges on the screen, one giving an equal (cf. Fig. 3)

Chapter I

The result of ventral root stimulation on contralateral motoneurones

Activity in motoneurones are known to influence motor nuclei located in the same and adjacent segments via the recurrent collaterals (RENSHAW 1941 ECCLES FATT and KOKETSU 1954 WILSON 1959) In the present investigation it was of importance to find out whether impulses in alpha efferents were also capable of influencing contralateral motoneurones

The curves in Fig 1 refer to an experiment on an acute low spinal cat On either side the monosynaptic reflex from BSt recorded in the S_1 ventral root was conditioned by single volleys in the contralateral combined L_7 and S_1 ventral roots at a strength maximal for the alpha *efferents* There was not a trace of an effect (□ ▲) whereas the usual long lasting Renshaw inhibition appeared with conditioning of the ipsilateral L_7 ventral root (× ●) Similar findings were made on test reflexes from the G nerve

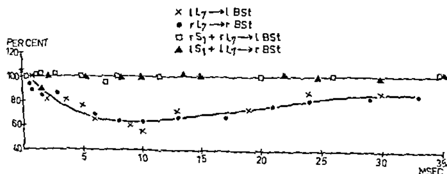


Fig 1 Effects of single cond tioning volleys ... its the unconditioned amplitude of the test reflexes Conditioned amplitudes of monosynaptic test reflexes expressed as percentage of control amplitudes are plotted as a function of the time interval between incoming conditioning and testing volleys which were recorded at the dorsal root entry zone Acute spinal (L_2) cat

Low threshold muscle afferents denotes group I muscle afferents and *high threshold muscle afferents* group II and III muscle afferents

The term *double reciprocal innervation* will be used to denote contralateral actions of opposite effect to the ipsilateral ones SHERRINGTON (1909 b) did not use the term entirely in this sense

Chapter II

Crossed spinal actions from low threshold muscle afferents

Introduction

The only previous investigation with modern electrophysiological techniques on crossed group I effects is that by PERL (1958 1959) On acute high spinal animals he found that small group I volleys from the Q Cr nerve inhibited contralateral Q Cr monosynaptic reflexes and at the same time facilitated the antagonist the contralateral BSt reflexes When the group I volley was increased he found crossed facilitation of the Q Cr and inhibition of the BSt reflexes A corresponding set of double reciprocal effects on BSt and Q Cr respectively were observed when conditioning the BSt nerve PERL suggested that the actions evoked by low threshold volleys might be due to activity in group Ia fibres and the additional actions by more high threshold afferents to Ib impulses

In the present investigation crossed group I effects were not found in acute spinal animals and in chronic low spinal animals it has usually been necessary to employ repetitive stimulation to reveal crossed group I actions In section A the criteria are given by which effects can be ascribed to impulses in group I fibres Section B deals with attempts to utilize the two components of the group I volley in this study of crossed connections The pattern of crossed group I effects is described in section C

Results

A Criteria for ascribing crossed effects by a train of volleys in muscle nerves to activity in group I muscle afferents

In a series of experiments on acute low spinal animals with prominent crossed actions from high threshold muscle afferents (described in chapter III) no or uncertain crossed effects were observed on single or repetitive stimulation of muscle nerves at group I strength The findings reported in this chapter were made on chronic spinal animals 2—6 weeks after transection of the cord in the upper lumbar region Even in these preparations single group I volleys had at most small crossed effects

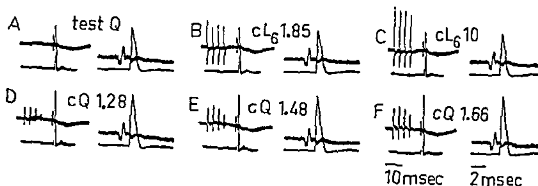


Fig 2 Effects of a train of conditioning volleys in the ventral L_6 root (B and C) and in the Q nerve (D—F) on the Q monosynaptic test reflexes recorded in the ventral root on the contralateral side The unconditioned test reflexes are shown in A The conditioning stimulus strengths relative to the threshold strength for the root and nerve are indicated in the records In each record the right traces were obtained simultaneously at faster sweep speed to show the height of the monosynaptic reflexes (lower traces) while the left traces show the time relation between the testing and conditioning incoming volleys recorded in the dorsal root entry zone (upper traces) The ventral roots L_5—S_1 were cut bilaterally All records were obtained from about 10 superimposed traces Chronic spinal cat

Experiments were also made on chronic spinal cats, 2—6 weeks after transection of the cord, and in these experiments a train of stimuli was given to the ventral roots Neither under these circumstances were there any effects on contralateral monosynaptic test reflexes as is illustrated in Fig 2 where the Q test reflexes were recorded in the L_6 ventral root (shown unconditioned in A) and conditioned with 4 volleys in the contralateral L_6 ventral root (B and C) In Fig 2 the actions are illustrated only at one interval from a series The stimulus strength of 1 85 times threshold for the ventral root (B) was chosen in order to activate the alpha fibres and 10 times threshold for activating the gamma fibres (C) (LEKSELL 1945) For comparison the lower row (D—F) shows the crossed actions by a similar train of impulses activating low threshold muscle afferents This action must be mediated through the dorsal roots as the ventral L_5—S_1 roots were cut The finding that there are no actions on contralateral motoneurones from antidromic activation of efferent fibres gives the methodological advantage that it is not necessary to section the ventral roots on the conditioning side when investigating the crossed effect of volleys in muscle afferents evoked by stimulation of a muscle nerve

Summary

In spinal cats antidromic volleys in efferent fibres had no effect on contralateral motoneurones tested on their monosynaptic reflex discharges

could be very brief less than 2 msec (cf Fig 3 G) This indicates a minimal pathway with two interneurones The duration of action evoked by 2 to 3 volleys was usually 20—30 msec

It is now necessary to discuss the criteria for ascribing these effects to group I afferents Even with single conditioning volleys this may be difficult since there is an overlap in threshold between group I and II fibres but the usage of repetitive stimulation gives a further complication

Fig 4 illustrates the customary procedure of measuring the actions with graded conditioning stimulation In each record is shown the effect of a conditioning train from the Q nerve on the contralateral Q test reflexes (upper traces) at the stimulus strength indicated and immediately below at the same sweep speed the conditioning train triphasically recorded at the dorsal root entry zone The lower traces in each record were obtained at faster sweep speed and are triphasic recordings of single volleys evoked at the strength indicated (left) and in combination with a supramaximal group I volley evoked in the refractory period of the first volley (right) In Fig 4 facilitation appears already in record B at a stimulus strength of 1 14 times threshold at which strength but a fraction of group I fibres are activated The facilitation is marked in D at 1 36 times threshold when the first stimulus activates only somewhat more than half of the group I afferents In G the strength of the first stimulus is almost maximal for group I but the increase in facilitation from D to G is comparatively small and there is only a slight increase when the strength is raised above group I (H and I) With effects evoked by such small group I volleys as in Fig 4 there can be little doubt that they are caused by impulses in group I afferents if subthreshold temporal facilitation in group II fibres can be excluded

For this purpose recordings were made from dorsal root filaments as is illustrated in Fig 5 with simultaneous recording from 2 filaments containing one (lower trace) and two (upper trace) afferents respectively from soleus A train of 4 stimuli was given to the soleus nerve The stimulus strengths indicated are expressed as multiples of the threshold strength for the fastest conducting fibre The three afferents conducted at velocities of 107 94 (upper trace) and 66 m/sec (lower trace) which according to HURSH (1939) correspond to fibre diameters of 18 16 and 11 μ The two coarse fibres are in the group I range and the thin one in the group II range The thresholds were 1 0 (A) 1 26 (E) and 1 79 (H) respectively The fibres were regularly activated by all stimuli (at a frequency of 250/sec) at a strength of 1 26 (E) 1 49 and 2 21 times threshold respectively It was found that with repetitive stimulation at the frequencies used group I and II fibres are recruited in essentially the same manner When the

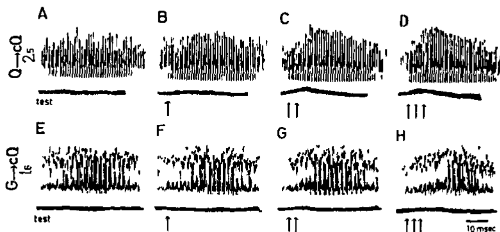


Fig 3 Crossed actions on the Q monosynaptic test reflexes (shown unconditioned in A and E) evoked by one (B and F), two (C and G) and three (D and H) conditioning volleys in the Q nerve (B—D) and the G nerve *(F—H) at maximal group I stimulus* strength for the nerves (2.5 and 1.6 times threshold respectively)

Each record consists of about 150 monosynaptic reflexes superimposed in one photographic exposure evoked at various intervals after the sweep start while the conditioning stimuli were fixed in time Arrows indicate time of arrival of the conditioning *group I volleys recorded in the dorsal root entry zone The lower trace is an integrated* recording of the monosynaptic discharges (Further explanation is given under methods) Chronic spinal cat

which could wax or wane and hence were difficult to analyse systematically Repetitive stimulation was tried for conditioning and was found to be much more effective as is illustrated in Fig 3 Records B—D show the effect of conditioning volleys in the Q nerve and records F—H in the G nerve on contralateral Q monosynaptic test reflexes (shown unconditioned in A and E) The conditioning strengths 2.5 (B—D) and 1.6 (F—H) times threshold for respective nerves was just maximal for group I on single volley stimulation In B a single conditioning volley in Q has some facilitatory effect but the effect increases considerably when two or three stimuli were given in C and D On the other hand there is no visible effect by a single conditioning group I volley in the G nerve (F) but facilitation appears with two conditioning volleys (G) and increases with the addition of a third volley (H) In excitable preparations large effects were often found with a conditioning train of 2—3 volleys but the actions could sometimes be increased when 4—6 volleys were employed

Under conditions when a single volley had no action the facilitatory effect evoked by the second volley appeared after a central delay which

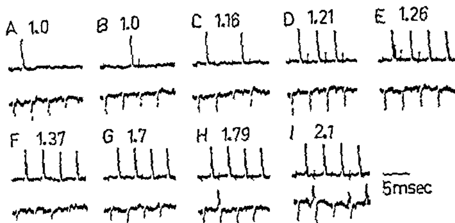


Fig 5 Simultaneous recording in two dorsal root filaments. *A conditioning train of 4 impulses was applied to the soleus nerve at indicated stimulus strengths expressed as multiples of the threshold for the fastest conducting fibre*

resemble group I fibres In G at 1 7 times threshold the *group II fibre* (lower trace) failed to be activated in 12 *trials* In H at 1 79 times thresh old it responded to the first stimulus in 6 trials *out of 12 (in 4 trials the* fibre responded only to the first stimulus *and in two trials* to the first and third stimuli) In the remaining 6 trials the fibre did not respond at all Similar findings *were also made* on slower conducting group II fibres Hence no evidence was obtained *suggesting subthreshold* temporal sum mation in group II fibres at the frequencies used for conditioning in this series of experiments

In many experiments recording was afterwards made from dorsal root filaments in order to measure *the threshold* of the group II fibres (cf Fig 6 7 and 16) All effects appearing below threshold for group II have been accepted as group I effects but with employment of repetitive stim ulation it has often been difficult *to decide* whether an effect appearing at or slightly above threshold for the *group II* was evoked by impulses in high threshold group I or low threshold group II fibres Even if the first stimulus in a train is slightly supramaximal for group I later stimuli may be submaximal in other *words the overlap* in threshold between group I and group II increases with repetitive stimulation

Fig 6 exemplifies crossed *actions* appearing at maximal group I *strength* but in this case it could be shown that the effect was caused by activity in group II fibres Two stimuli (8 msec apart) to the BSt *nerve were used* to condition the test reflex from the contralateral BSt In B at 1 41 times threshold the double volley test upper right traces in each record) shows

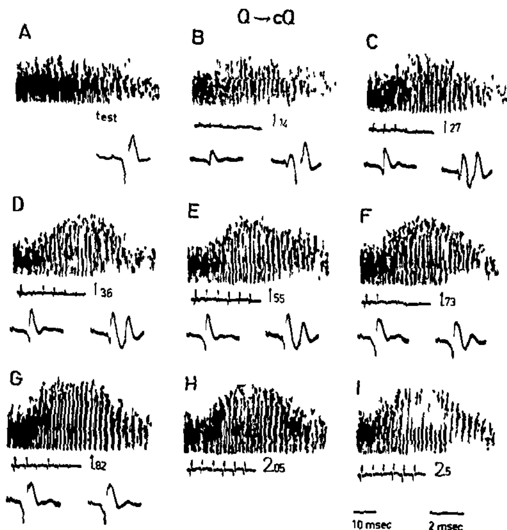


Fig 4 Actions on the Q monosynaptic test reflexes evoked by a train of impulses in the contralateral Q nerve Stimulus strengths expressed in multiples of threshold for the nerve are indicated The incoming conditioning volleys were triphasically recorded in the dorsal root entry zone (middle traces) with the same time base as the test reflexes in the upper traces Triphasic recordings at faster sweep speed of single volleys evoked at the indicated stimulus strengths are given in lower left traces and in combination with a supramaximal group I volley (shown unconditioned in A) evoked in the refractory period of the first volley in lower right traces The records of dorsal root volleys are superimposed traces *Procedure and conventions as in Fig 3 Chronic spinal cat*

strength was at threshold for the fibre *investigated it was activated only* by the first stimulus (A, E and H), only very exceptionally by any of the others *(B) It is important that group II fibres in this respect closely*

consisted of Ia fibres and the slow of Ib fibres. Later investigations have corroborated this hypothesis although overlapping of the two groups has been found (LAPORTE and BESSOU 1957, ECCLES et al. 1957). In the present context it was naturally of interest to distinguish between connections from Ia and Ib fibres and considerable attempts have been made to utilize the threshold separation between the slow and fast components of the group I volley. In this series of experiments the crossed actions of thigh muscles displaying this separation were studied, particularly from the Q nerve which regularly gave large crossed actions. 12 experiments were made but in some of these the separation of the two components was not marked, neither with respect to threshold nor conduction velocity.

It is, however, obvious that the employment of repetitive stimulation gives a severe limitation because of changes in threshold with successive stimuli: the first stimulus of a train may be supraliminal for the Ib component and later stimuli may not suffice to excite all fibres of the Ia component.

In Fig. 4, already discussed in section A, which illustrates the action from Q to contralateral Q with graded conditioning stimulation, there is a clear-cut facilitation in record B where the first stimulus *in the train* gives a small Ia volley. The facilitation increases considerably in C and D where the first volley in the train is an almost maximal Ia volley, whereas there is very little sign of activation of the Ib component. The surface lead recording in B—D also shows that later volleys in the train decrease markedly and represent only a fraction of the Ia volley. When the stimulus strength was increased further in E—G to activate also the Ib component there was only a small increase in facilitation of the contralateral Q monosynaptic reflex. This finding would suggest that the facilitation was contributed mainly by fibres of the Ia component. Similarly in Fig. 15 the facilitation from Q on the contralateral DP monosynaptic reflexes appears with a very low threshold group I volley, and as in Fig. 4 it seems to be contributed by the Ia component.

Although it was regularly observed that this contralateral facilitation from Q appeared at very low stimulus strengths, it was by no means always possible to ascribe it exclusively to the Ia component. For example in Fig. 7 (right records in each column) and Fig. 8, both showing effects from Q to contralateral Q, it is not possible to state whether a major part of the facilitation at medium group I stimulus strength is due to activation of fibres of the Ia component in the latter part of the train or to fibres of the Ib component in earlier volleys.

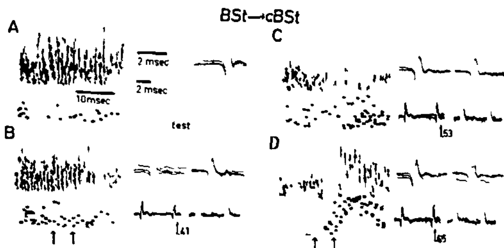


Fig 6 Actions of two conditioning volleys from BSt (B—D) on the contralateral BSt monosynaptic test reflexes shown unconditioned in A The time of arrival at the dorsal root entry zone of the conditioning BSt volleys is indicated by arrows only in the *lowermost records (B and D) Triphasic recordings at faster sweep speed are shown in* lower middle traces and monophasic recordings, obtained in the end of the experiment in lower right traces The double volley test, upper middle and upper right traces was performed as described in Fig 4 For procedure and conventions see also Fig 3 Chronic spinal cat Some records are retouched

that the stimulus evoked an almost maximal group I volley but there was no crossed action When the stimulus was raised to 1 53 times threshold maximum group I (C) there was some facilitation, and at 1 65 times threshold slightly supramaximal for group I, (D) the facilitation was prominent This facilitation occurred after so short latency that its onset and early part must have been due to the first conditioning volley Since the main increase in facilitation occurred from C to D, and since subsequent monophasic recording from a dorsal root filament revealed *a trace of* activity in group II fibres in C and a considerable activity in D (lower trace to the extreme right in each record of Fig 6) it can be concluded that *the facilitation in Fig 6 was caused by impulses in group II fibres*

B *Attempts to separate group Ia and Ib effects*

It is well known that among group I fibres are *afferents from two recep*tive systems Ia fibres with annulo spiral endings on muscle spindles and *Ib fibres from Golgi tendon organs* BRADLEY and ECCLES (1953) drew *the attention to the fact that the group* I volleys in nerves from thigh muscles often have two components and suggested that the first component

consisted of Ia fibres and the slow of Ib fibres Later investigations have corroborated this hypothesis although overlapping of the two groups has been found (LAPORTE and BESSOU 1957 ECCLES et al 1957) In the present context it was naturally of interest to distinguish between connections from Ia and Ib fibres and considerable attempts have been made to utilize the threshold separation between the slow and fast components of the group I volley In this series of experiments the crossed actions of thigh muscles displaying this separation were studied particularly from the Q nerve which regularly gave large crossed actions 12 experiments were made but in some of these the separation of the two components was not marked neither with respect to threshold nor conduction velocity

It is however obvious that the employment of repetitive stimulation gives a severe limitation because of changes in threshold with successive stimuli the first stimulus of a train may be supraliminal for the Ib component and later stimuli may not suffice to excite all fibres of the Ia component

In Fig 4 already discussed in section A which illustrates the action from Q to contralateral Q with graded conditioning stimulation there is a clear cut facilitation in record B where the first stimulus in the train gives a small Ia volley The facilitation increases considerably in C and D where the first volley in the train is an almost maximal Ia volley whereas there is very little sign of activation of the Ib component The surface lead recording in B—D also shows that later volleys in the train decrease markedly and represent only a fraction of the Ia volley When the stimulus strength was increased further in E—G to activate also the Ib component there was only a small increase in facilitation of the contralateral Q monosynaptic reflex This finding would suggest that the facilitation was contributed mainly by fibres of the Ia component Similarly in Fig 1b the facilitation from Q on the contralateral DP monosynaptic reflexes appears with a very low threshold group I volley and as in Fig 4 it seems to be contributed by the Ia component

Although it was regularly observed that this contralateral facilitation from Q appeared at very low stimulus strengths it was by no means always possible to ascribe it exclusively to the Ia component For example in Fig 7 (right records in each column) and Fig 8 both showing effects from Q to contralateral Q it is not possible to state whether a major part of the facilitation at medium group I stimulus strength is due to activation of fibres of the Ia component in the latter part of the train or to fibres of the Ib component in earlier volleys

One source of error in an attempt to interpret these actions in terms of effects by Ia and Ib fibres is the 'contamination' between the two groups a small number of Ib fibres may be contained in the fast component of the group I volley and vice versa, some Ia fibres in the slow component (LAPORTE and BESSOU 1957, ECCLES et al 1957) This possibility is of importance since *repetitive stimulation may tend to increase the effect* from a small number of *fibres The findings in Fig 7 are of interest in* this respect In this experiment the actions from Q on the contralateral monosynaptic reflexes from Q (right records in each column) were compared with the actions on the ipsilateral monosynaptic reflexes from med G (left records in each column) In both cases a train of 4 volleys was used for conditioning and at each strength the effects were alternately recorded on both sides At a strength of 1 15 times threshold record C there is contralateral facilitation and ipsilateral inhibition These effects increase at 1 24 times threshold in D and at 1 36 times threshold in E where the double volley test indicates that the strength is maximal for the Ia but subliminal or at threshold for the Ib component In F and G when the conditioning strength was raised to activate fibres of the slow component there is a considerable increase of the ipsilateral inhibition to med G *and also some augmentation in contralateral facilitation* to Q The *only known connection by which group I* afferents from Q ipsilaterally can inhibit the monosynaptic reflex from med G (recorded in S_1) is by the Ib pathway and the inhibition in C—E may be due to Ib fibres in the fast component and if so impulses in these afferents might also be responsible for the crossed effects However, the possibility still remains

Fig 7 The actions of a train of volleys in the Q nerve (B—G) were alternately measured on ipsilateral monosynaptic reflexes from the med G nerve recorded in the S_1 ventral root *(left records in each column) and on contralateral monosynaptic reflexes from* the Q nerve recorded in the L_6 ventral root (right records in each column) The unconditioned test reflexes are shown in A In the records of Q → cQ the triphasic recordings (left traces below the integrated curves) show the time of arrival of the conditioning volleys in the records of Q → i med G *the time of arrival is indicated only by* arrows in the lowermost records (D and G) In B—K (lowermost left traces in B—G) the train of conditioning volleys was monophasically recorded afterwards In F—K at faster sweep speed single volleys are also shown monophasically recorded at the given *stimulus strength*

The *crossed excitatory* action from Q at the stimulus strengths used in F—G was sufficient to evoke reflex discharges in the L_6 ventral root and hence also recorded at all time intervals when these motoneurones were not refractory from the test reflex These discharges in the ventral root appear as a *white shade at the base of the upper right traces in records* F—G at the time of the maximal facilitatory effect on the test reflexes Procedure and conventions as in Fig 3 4 and 6 Chronic spinal cat Some records are retouched

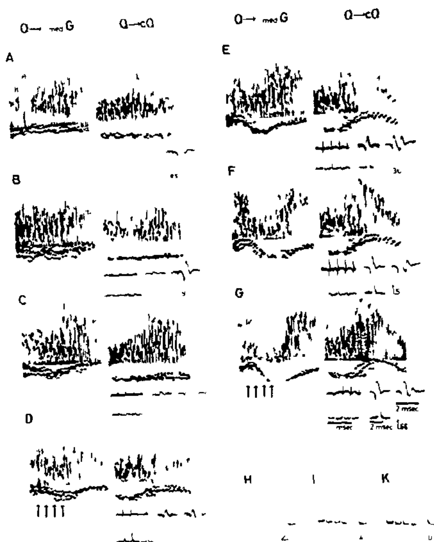


Fig 7

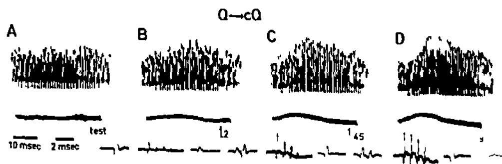


Fig 8 Actions on the Q test reflexes evoked by volleys in the contralateral Q nerve The same chronic spinal cat as in Fig 9 10, 12 and 13 Procedure and conventions as in Fig 3 and 4

that the actions are evoked by different fibre systems because they may have different interneuronal linkages, if the linkage of the crossed path was weaker than the ipsilateral, impulses in a larger number of afferents would be required A more definite suggestion regarding the source of the actions evoked by impulses in low threshold muscle afferents is not permissible on the basis of the present experiments

C *Pattern of crossed group I actions*

Effects of repetitive stimulation of group I afferents from various thigh and leg muscles have been examined on monosynaptic reflexes from contralateral extensors and flexors For reasons discussed in the last section, no attempt will be made to distinguish between effects by I a and I b impulses

a) To extensors

Crossed facilitatory action between Q has already been illustrated in Fig 4 and 7 and it will now be shown that crossed facilitatory actions are common between extensors No inhibitory interaction was ever found between extensors Fig 8—10 are from the same experiment and illustrate facilitation of the Q monosynaptic reflex by short group I trains from the contralateral Q nerve (Fig 8 B—D), from the contralateral G nerve (Fig 9 F and G), and from the contralateral FDL nerve (Fig 10 B and C) In this experiment facilitation of Q monosynaptic reflexes was also evoked by a train (at just maximal group I strength) from the plantaris nerve Sometimes the effect from ankle and toe extensors to Q was quite marked at

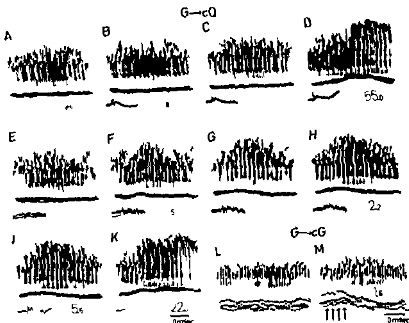


Fig 9 Actions evoked by volleys in the G nerve on contralateral Q (B—K) and contralateral G (M) test reflexes shown unconditioned in A and L respectively The same chronic spinal cat as in *Fig 8 10 12* and *13* Procedure and conventions as in Fig 3 and 4

the beginning of the experiment but decreased gradually so that at the end of the experiment *only the effect* from Q to contralateral Q remained In other less excitable *preparations* the only crossed group I action which could be obtained at all was from Q to contralateral Q

Similar crossed facilitation of *monosynaptic* reflexes was also found from other extensor nerves Record M in Fig 9 shows the effect by a group I train in the *G nerve* on the contralateral G test reflexes and in this experiment the same conditioning *train from* G also facilitated the contralateral *FDL test reflex (not illustrated)* These effects from G to contralateral G and FDL were small but recorded at the end of the experiment Crossed group I *facilitation between* ankle and toe extensors was not particularly prevalent but of the same order of magnitude as that found from these muscles to the Q *test reflex* (cf table I p 31) On the other hand considerable facilitation has been observed from Q to contralateral G The effects from the Q nerve *were often* larger and in less excitable pre



Fig 10 Actions evoked by volleys in FDL on contralateral Q test reflexes The same chronic spinal cat as in Fig 8 9 12 and 13 Procedure and conventions as in Fig 3 and 4

Fig 11 Actions evoked by volleys in Q on contralateral G monosynaptic reflexes Chronic spinal cat Procedure and conventions as in Fig 3 and 4 Some records are retouched

parations this latter effect has been observed when crossed interaction between leg muscles was lacking Fig 11 shows the effect from Q to contralateral G There is a weak effect in B at a strength of 1 25 times threshold and a marked increase with a more complete recruitement of group I fibres

in C—E The triphasic recordings from the dorsal root entry zone show that the Q volley displayed separation in I a and I b components The effect in Fig 11 appears mainly when the slow component was included in the train In the same experiment facilitation from Q to contralateral Q was found at a much lower stimulus strength which activated only a small part of the fast group I component in much the same way as has been illustrated in Fig 4 This may suggest that different afferent systems were responsible for facilitation of Q and G test reflexes an alternative explanation could be that both effects originated from the same afferent systems but that the linkage of the former pathway was stronger and hence impulses in fewer afferents were required In another experiment a similar comparison of the appearance of facilitation of Q and G test reflexes in relation to the size of the conditioning group I train from contra lateral Q did not reveal a difference of the type described above

To summarize crossed facilitatory group I interaction between exten *sors is common and widespread (cf table I p 31) Effects from* Q are more marked than from the other extensors investigated and Q is also more receptive at least with respect to effects from the contralateral Q

There are also crossed effects by group I volleys from flexors on test reflexes from extensors Systematic investigations have been made of crossed actions contributed from BSt and DP and important differences between the crossed group I effects on extensor test reflexes were found from these two nerves Altogether effects from BSt were rare but when observed inhibitory whereas the effects from DP were common and al ways facilitatory

Fig 12 illustrates effects from BSt to contralateral Q In G and H at a strength submaximal to group I a brief train of volleys inhibits the contralateral Q test reflex and the effect increases somewhat in I at a strength of 2 33 times threshold which was slightly supramaximal for group I On further increase of the stimulus strength (L and M) this in hibition decreases and reverses to facilitation (N) The facilitatory action in L—N by impulses in high threshold afferents would be expected since single condition ng stimuli of the same strengths give this effect (D and E) whereas a single group I volley has no significant inhibitory effect (B and C) The inhibitory group I effect from BSt is probably not limited to Q in two cases there was probably slight group I inhibition of the contralateral G test reflex likewise with reversal to excitation when high threshold afferents were activated BSt provided an exception in giving opposite actions from low and from high threshold muscle afferents Volleys in low and high threshold muscle afferents from all other nerves evoked the same modality of crossed action (cf Fig 9 and 13)

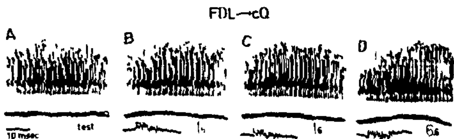


Fig 10 Actions evoked by volleys in FDL on contralateral Q test *reflexes* The *same* chronic spinal cat as in Fig 8 9 12 and 13 Procedure and conventions as in Fig 3 and 4

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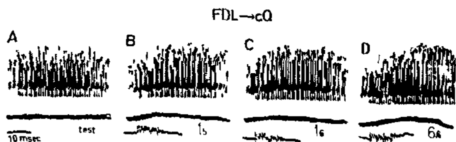


Fig 10 Actions evoked by volleys in FDL on contralateral Q test reflexes The same chronic spinal cat as in Fig 8 9 12 and 13 Procedure and conventions as in Fig 3 and 4

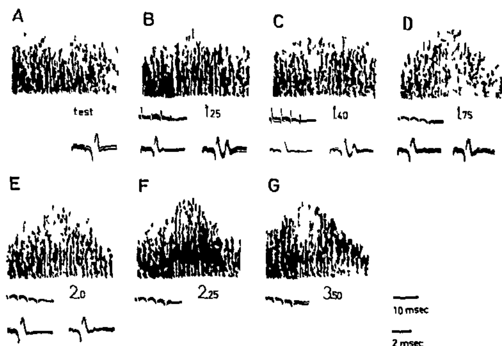


Fig 11 Actions evoked by volleys in Q on contralateral G monosynaptic reflexes Chronic spinal cat Procedure and conventions as in Fig 3 and 4 Some records are retouched

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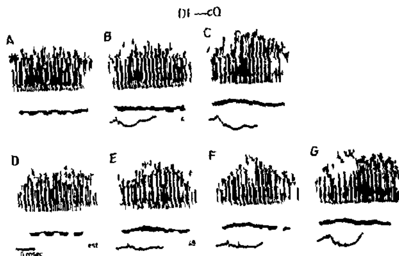


Fig 13 Actions evoked by volleys in DP on contralateral Q monosynaptic test reflexes The same chronic spinal cat as in Fig 8—10 and 12 Procedure and conventions as in Fig 3 and 4

From DP on the other hand there was facilitation to contralateral Q (Fig 13) and in contrast to the effect from BSt this effect was found frequently It seems likely that the effect in *Fig 13 E* and *F* was evoked by impulses in very low threshold group I fibres but the detailed interpretation is somewhat complicated by the fact that the DP nerve at low stimulus strengths did not follow *repetitive stimulation* very well as often happened with this nerve (cf triphasic recordings in Fig 13 14 and 17) Group I volleys from DP have also been observed to *facilitate* contralateral G but this effect was *much* smaller than to contralateral Q

Both from BSt and DP the largest crossed group I actions were found on the Q test reflex It should however be remembered that Q is not a uniform muscle V Cr (vasto crureus) is a pure knee extensor but R (rectus) being a double joint muscle can in addition act as a hip *flexor* The crossed effect from DP is *presumably* associated with the extensor function because when monosynaptic reflexes from R and *V Cr* were investigated separately both were facilitated in much the same way as is illustrated in Fig 14 (*with four different* strengths of DP stimulation) In the same experiment it *was also found* that a conditioning group I train from the Q nerve facilitated contralateral R and V Cr reflexes in parallel as in Fig 14

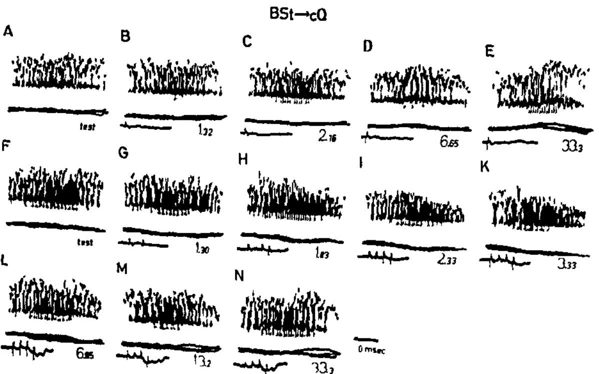


Fig 12 Actions evoked by volleys in BSt on contralateral Q monosynaptic reflexes The same chronic spinal cat as in Fig 8—10 and 13 Procedure and conventions as in Fig 3 and 4

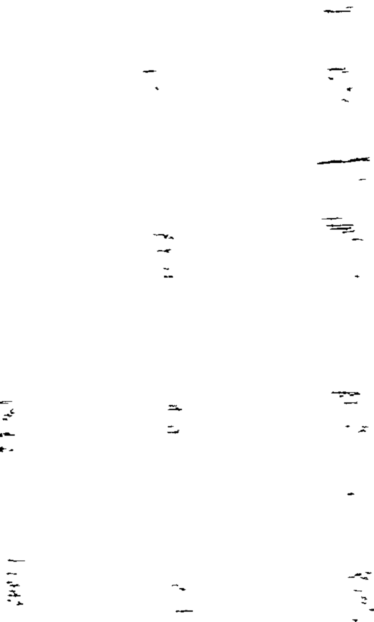


Fig 15

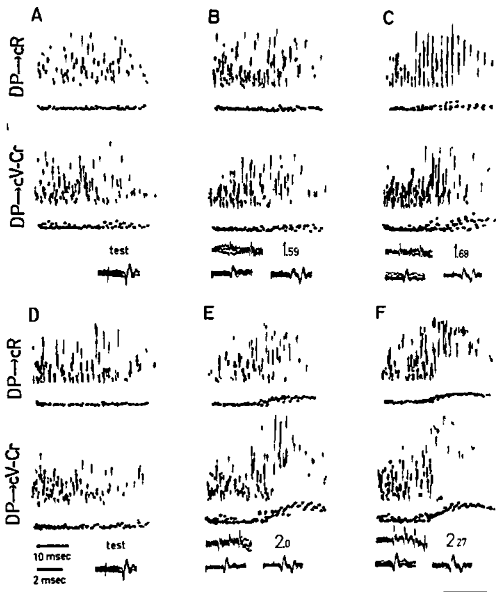


Fig 14 Crossed effects evoked by volleys in the DP nerve on monosynaptic reflexes from R and V Cr as indicated At each conditioning strength alternate recording was made of the effect on both test reflexes Chronic spinal cat Procedure and conventions as in Fig 3 and 4 Some records are retouched

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Fig 15 Actions on DP monosynaptic reflexes evoked by volleys in the contralateral Q nerve Chronic spinal cat Procedure and conventions as in Fig 3 and 4

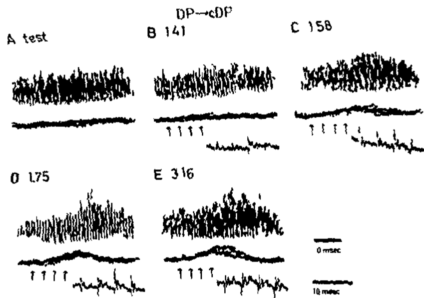


Fig 17 Actions evoked by volleys in the DP nerve on the contralateral DP monosynaptic reflexes The train of conditioning volleys was triphasically recorded at the dorsal root entry zone but at faster sweep speed (lowermost traces) Otherwise the procedure and conventions were as in Fig 3 Chronic spinal cat

facilitation of the contralateral DP reflexes Both effects appear in B at a strength of 1 24 times threshold and the differences in growth between the two crossed actions with increasing strength (C—E) is not sufficiently marked to justify a suggestion that different receptive systems were responsible The question may be raised whether the difference in modality of action can be related to the fact that Q is the contralateral antagonist of BSt whereas DP operates at another joint No evidence in this direction is given by the group I actions to DP from the crossed antagonist FDL A short train from this nerve evoked at maximal group I strength produced facilitation probably due to activity in group I fibres

Of crossed connections between flexors the only more frequent effect was facilitation to DP by a maximal group I train from contralateral DP which effect presumably could be ascribed to group I afferents This is illustrated in Fig 17 There is probably no effect in B at 1 41 times threshold but a marked effect in C at 1 58 and in D at 1 75 times threshold which was just maximal for group I The increment in effect is probably

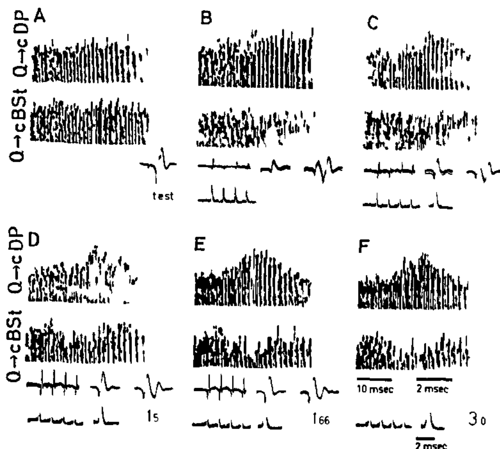


Fig 16 Effects by volleys in the Q nerve on the contralateral DP test reflexes and on the contralateral BSt monosynaptic test reflexes as indicated At each conditioning strength alternate recording was made Chronic spinal cat Procedure and conventions as in Fig 3 4 6 and 7

b) To flexors

Systematic investigations were made only of the effects on BSt and DP monosynaptic reflexes The most marked effects were found from the Q nerve Fig 15 shows the frequently occurring crossed facilitation from Q to contralateral DP As has already been discussed in section B the effect appears with small group I volleys in B and C and does not increase significantly when slower conducting group I fibres are included in the conditioning train (F—H)

Test reflexes from BSt on the other hand were inhibited by a group I train from the contralateral Q nerve This is illustrated in Fig 16 where at each conditioning strength this inhibitory effect is compared with the

the group I volley to disclose I a and I b effects (cf BRADLEY and ECCLES 1953 ECCLES et al 1957 LAPORTE and BESSOU 1957) The employment of repetitive stimulation undoubtedly complicates the interpretation because it gives a threshold overlap between the fast and the slow component (cf section A) Furthermore it has recently been shown that some synaptic actions of I b impulses are considerably enhanced when repetitive stimulation is employed while synaptic actions from I a fibres are decreased (CURTIS and ECCLES 1960 ECCLES HUBBARD and OSCARSSON 1961) Nevertheless it seemed worth while to try this technique The results were puzzling facilitation from Q to contralateral Q appeared with stimulation of low threshold group I fibres (cf Fig 4) and did not always increase when the I b component was included In other experiments the I b component probably also contributed facilitation This finding may indicate that crossed I a facilitation from Q to contralateral Q can occur but at present it cannot be excluded that the facilitatory effect of the I a volley is due to contamination of I b fibres in this component (cf control experiment in Fig 7)

Crossed effects which could be ascribed to impulses in group I afferents are summarized in table 1 Facilitatory interaction between extensors was common The most marked effects were observed from the Q nerve and reached not only contralateral Q but also all other extensors tested In no case were dual crossed actions by impulses in group I afferents observed

The crossed group I facilitatory effect from extensors to extensor test reflexes was not regularly reciprocated by crossed inhibition from flexors but inhibition from BSt to contralateral Q has been observed Group I trains from the DP nerve on the other hand regularly facilitated the contralateral Q test reflex In their receptiveness BSt and DP also displayed characteristic differences (cf table 1) DP test reflexes were frequently group I facilitated from the contralateral Q nerve whereas BSt test reflexes were inhibited These findings suggest that there are important differences in crossed connections to knee and ankle flexors It is not known whether these differences have any relation to the fact that tibialis anterior has two components with different contraction time (GORDON and PHILLIPS 1953)

There are several discrepancies between the present results and those reported by PERL (1958) In the present series of experiments fine gradation of stimulus strength did not reveal dual crossed group I actions PERL (1958) on the other hand described a weak short lasting (3 msec) inhibition to contralateral V Cr by a single small group I volley from V Cr followed by a prominent long lasting (10 msec) facilitation when a larger group I volley was used for conditioning To the nucleus of the

mainly due to the more effective activation of later volleys in the train (cf triphasic recordings lower traces) From BSt to contralateral BSt there were never any effects which could be ascribed to impulses in group I afferents (cf Fig 6)

A train of group I volleys particularly in the Q nerve, sometimes evoked a discharge in contralateral motoneurones (Fig 7) It was however, controlled that all the actions described in this chapter were not secondary effects to a crossed reflex discharge (cf RENSHAW 1941, ECCLES et al 1954 WILSON 1959, WILSON, TALBOT and DIECKE 1960), since they were also found when there was no sign of crossed reflex discharges

Table 1
Actions evoked by a train of conditioning group I volleys on contralateral test reflexes

Contralateral test reflex	Conditioning nerve					
	Q	G	Pl	FDL	BSt	DP
Q	+	+	+	+	–	+
G	+	+		(+)	(–)	(+)
FDL	+	+		(+)		
BSt	–					
DP	+			(+)		(+)

Table 1 summarizes the crossed actions evoked by a train of impulses which could be ascribed to group I afferents + denotes facilitation of the contralateral test reflex and — inhibition Actions within *brackets indicate* effects which either were weak or evoked at just maximal group I strength without further analyses to ascertain the origin of the effects ascribed above Even fine gradation of conditioning stimulus strength never revealed any signs of dual actions by impulses in group I afferents

Discussion

In the present investigation on acute low spinal cats no crossed group I effects on contralateral monosynaptic test reflexes were discernable on single or repetitive stimulation Even in chronic spinal animals single conditioning group I volleys gave no or only small effects whereas a brief train of stimuli produced considerable crossed actions Repetitive stimulation provides a complication *but the criteria for ascribing effects* to group I afferents have been given in section A

Any relevant discussion of the functional organization of group I connections must consider the I a and I b systems separately In many experiments of this series attempts were made to utilize the two components of

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Attempts to utilize the fast and slow components of the group I volleys to distinguish between effects evoked by impulses in I a and I b fibres were not successful (section B) Experiments with fine gradations of stimulus strength did not give evidence of dual crossed group I effects

The pattern of crossed group I effects is described in section C and summarized in table 1 Crossed facilitatory interaction between extensors was common particularly large effects were evoked from and received by Q Crossed effects were also found from flexors to extensors there was facilitation from DP to Q and occasionally inhibition from BSt to Q

Of crossed group I effects to flexors the most marked were facilitation from Q to DP and inhibition from Q to BSt

antagonist (BSt) PERL found though less regularly a reciprocal set of actions These actions were assumed to be I a and I b effects respectively If so repetitive conditioning volleys at frequencies employed in this investigation would for two reasons favour the appearance of these latter actions partly because of the above mentioned difference in synaptic transmittability of I a and I b impulses (CURTIS and ECCLES 1960 ECCLES et al 1961) and partly because of the difference in duration and magnitude of the inhibitory and facilitatory actions described by PERL PERL (1958) also claimed two sets of crossed reciprocal group I actions from BSt Of these four effects the only one found by repetitive stimulation was the inhibition from BSt to contralateral Q

It is of interest to correlate these findings with previous experiments on reflex contractions in muscles The most prominent crossed spinal reflex between knee extensors is Philippson s reflex which is the crossed correlate to the ipsilateral lengthening reaction (SHERRINGTON 1909 a) It is generally assumed that the lengthening reaction is a I b effect (cf GRANIT 1955) and it has also been suggested that Philippson s reflex is caused by impulses in I b fibres (MOUNTCASTLE 1956 PERL 1958) In SHERRINGTON s experiments there was usually relaxation of the contralateral Q during the shortening reaction which is generally accepted as a I a effect but there was sometimes contration of the contralateral Q The possibility must however be kept in mind that these effects may be due to other receptive systems possibly co activated with I a when Q was stretched

It is important to keep in mind that Q is a composite muscle and that fractions of it may receive different crossed connections (cf ECCLES and GRANIT 1929) However in the present investigation the effect of conditioning volleys from Q and DP on contralateral test reflexes from R and V Cr closely resembled each other Another possibility has not been excluded crossed connections to the vasti and crureus may be different the former muscles being engaged in phasic movements and the latter in posture where a crossed I a excitation from Q to crureus could be purposeful in standing Furthermore there is also the possibility that on the conditioning side the various fractions of Q may have different connections to one and the same contralateral nucleus

Summary

Crossed effects of volleys in group I muscle afferents on monosynaptic test reflexes have been investigated in unanaesthetized cats In acute low spinal cats single group I volleys or a train of group I volleys had no effect

In chronic spinal cats single group I volleys gave only small effects but a train of volleys gave large effects The criteria for ascribing these effects to group I afferents have been given in section A

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Chapter III

Crossed spinal actions from the flexor reflex afferents

Introduction

Group II and III muscle afferents, high threshold joint afferents and skin afferents have been denoted the flexor reflex afferents (FRA) (ECCLES and LUNDBERG 1959 a, HOLMQVIST, LUNDBERG and OSCARSSON 1960) because in spinal preparations they evoke prominent ipsilateral actions in motoneurones in conformity with the flexion reflex (SHERRINGTON 1910) namely *excitation of flexor and inhibition of extensor motor nuclei* (cf also LLOYD 1943, BROCK, ECCLES and RALL 1951, LAPORTE and LLOYD 1952, ECCLES and LUNDBERG 1959 a).

The question now arises whether these afferent fibre systems also evoke crossed actions in accordance with the crossed extensor reflex appearing concomitantly with the ipsilateral flexion reflex. According to SHERRINGTON (1909 a, 1910) nociceptive stimulation as well as electrical stimulation of skin and muscle nerves giving an ipsilateral *flexion reflex* can evoke a movement of the fellow hindlimb with extension of the hip, knee and ankle. *The muscles contracting in the crossed extension reflex* were those relaxing in the ipsilateral flexion reflex, i e the extensors. Other muscles which relaxed in the crossed extensor reflex were those contracting in the ipsilateral flexor reflex, i e the flexors. The threshold for these crossed actions were sometimes about the same as for the ipsilateral reflex but occasionally considerably higher (cf also MC COUCH, SNAPE and STEWART 1935). The latency of the crossed action (40—100 msec) was *invariably longer than that of the ipsilateral reflex* (8—10 msec) and sometimes considerably delayed. In addition the contralateral reflex was characterized by a gradual onset and development (LIDDELL and SHERRINGTON 1923 a, b, CREED et al 1932).

An investigation with the aim to correlate various crossed actions to certain muscle afferent fibre systems has been made by PERL (1958). He found a complex pattern of actions from high threshold muscle afferents: the BSt monosynaptic test *reflex was inhibited* by a group II volley applied to the contralateral BSt, but the action reversed when thinner fibres were included in the conditioning volley. The most prominent action to the

Y Cr and G test reflexes was a longlasting facilitation evoked by activity in the thinnest myelinated fibres of flexors as well as extensors of the knee and ankle PERL (1957) also described two sets of double reciprocal action by impulses in cutaneous nerves A volley involving only coarse (14—6 μ) fibres resulted in excitation (central delay 3 msec) of the contralateral flexors at the knee and ankle and inhibition of extensors whereas activity in thinner cutaneous fibres (6—2 μ) gave a late (central delay 6—30 msec) prolonged inhibition to contralateral flexors and a corresponding late facilitation to contralateral extensors

This chapter deals with the crossed effects by impulses in the FRA in acute spinal and in chronic low spinal cats

Results

A *High threshold muscle afferents*

In the previous chapter the attention was drawn to the fact that in chronic spinal cats a single volley usually did not evoke any crossed effects as long as only group I fibres were activated but when the stimulus strength was raised to activate thinner fibres crossed effects appeared (cf Fig 9 12 and 13) Similar findings were also made in acute spinal animals The main investigation on crossed actions from high threshold afferents was performed on acute spinal animals and findings on chronic spinal animals will be summarized at the end of this chapter It could be excluded that the actions to be described were secondary effects to crossed reflex discharges (RENSHAW 1941 ECCLES et al 1954 WILSON 1959 WILSON et al 1960)

No essential difference was noted between crossed actions in animals with the cord transected in Th— L and those with a medial lesion at obex (cf table 2 A p 41 figures within brackets represent the number of experiments with lesion at obex) A comparison has also been made of the crossed actions found after a lesion at obex and after subsequent transection of the cord in the upper lumbar region and it was found that the latter section did not alter the results For this reason the results obtained in these two types of [illegible]

Fig 18 illustrate [illegible]
nerve on the DP te[illegible] [illegible] times threshold which was maximal for group I there was no effect (unconditioned test reflexes shown in A) In D at a strength of 4 7 [illegible]

[illegible] (1959 a) group II fibres [illegible] d up to 8—10 times threshold while group III fibres are

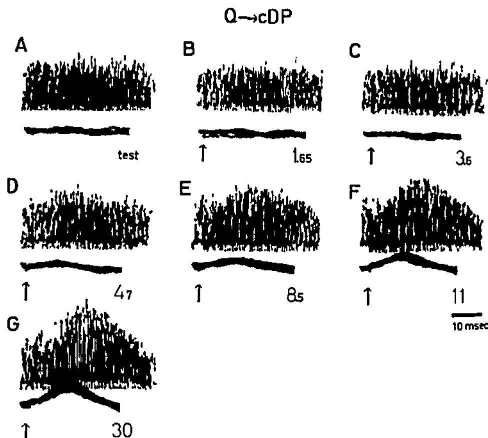


Fig 18 Actions evoked by volleys in the Q nerve on the contralateral DP monosynaptic test reflexes Acute spinal (L_2) cat the same as in Fig 19 Procedure and conventions as in Fig 3

activated at higher stimulus strengths hence the effect in D can be ascribed to group II afferents and the increase in facilitation from E and F to G to impulses in group III fibres It is of special importance that also group II volleys from the G nerve have been found to give significant crossed actions (cf Fig 20 A and 21 F) because the quantitative identification of group II fibres as spindle afferents (HUNT 1954) was made on gastrocnemius soleus afferents There was usually as in Fig 18 an increase of crossed actions when the stimulus strength was raised from a value activating most of the group II fibres to one activating group III fibres However, sometimes a group II volley did not give any detectable crossed actions In such cases group III volleys usually contributed large effects On the other hand in preparations with pronounced crossed actions by group II fibres, evoked at a conditioning strength of 5 times threshold, there

was only a small augmentation of the crossed facilitation when the conditioning volley was raised to include fibres in the group III range (cf Fig 20 B D and F) In no case did low threshold group II volleys and group III volleys evoke actions of opposite modality (i e excitation or inhibition)

The method employed for recording in Fig 18 is less suitable for latency measurements In this respect the technique to record the effect at fixed intervals is preferable In excitable preparations there are often inhibitory or excitatory actions when the group I component of the conditioning volley preceded the testing by 5 msec at the dorsal root entry zone Subtracting 1—2 msec for slower conduction velocity leaves about 3—4 msec for the central transmission Intracellular recordings have been made of crossed actions from high threshold muscle afferents and in these experiments the minimal central latency for EPSPs was 3 5 msec (HOLMQVIST and LUNDBERG unpublished) These delays suggest transmission through several interneurones However this central delay was often some msec longer The duration of crossed actions varied with the condition of the preparation and the stimulus strength used but the action often lasted about 25 msec This initial effect was sometimes followed by a prolonged reversed action which was not systematically analysed

High threshold muscle afferents from various muscles evoke in the same contralateral motor nucleus very similar actions a feature also found for their actions on ipsilateral motor nuclei (ECCLES and LUNDBERG 1959 a) Fig 19 is from the same experiment as Fig 18 and shows that single volleys in high threshold afferents from flexors (B and C) evoke a facilitation of the contralateral DP resembling the one evoked from Q (Fig 18) and from other extensors (Fig 19 D—F) In 22 experiments there were only two exceptions to this rule in both cases volleys in high threshold afferents from Q gave different crossed actions than corresponding volleys from other muscle nerves

The different extensor nuclei investigated (Q G Pl and FDL) almost invariably received the same modality of crossed action from the high threshold muscle afferents The only crossed flexor nucleus regularly investigated was BSt but when DP occasionally was tested its receptiveness was found to coincide with that of BSt (cf table 2 A p 41) It should however be observed that the receptiveness of DP was never investigated in an experiment in which BSt was inhibited from high threshold muscle afferents

In spinal cats in good condition volleys in the FRA usually excite flexor nuclei and inhibit extensor nuclei on the ipsilateral side (ECCLES and

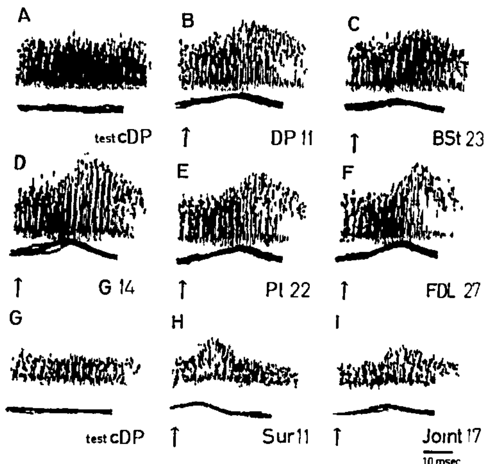


Fig 19 Crossed actions on DP monosynaptic test reflexes shown unconditioned in A and G evoked by volleys in the various nerves indicated Records G—I were taken with lower amplification than records A—F The same acute spinal (L_2) cat as in Fig 18 Procedure and conventions as in Fig 3

LUNDBERG 1959 a) Crossed actions from the FRA in accordance with the scheme of double reciprocal innervation i e excitation to extensor and inhibition to flexor nuclei, has also been observed This is exemplified by the experiment illustrated in Fig 20 in which volleys in high threshold muscle afferents gave excitation to the contralateral extensor nuclei G (curves in A—C) and Pl (curves in D), while the contralateral flexor nucleus BSt received inhibition (curves in E—G)

The pattern of crossed actions from high threshold muscle afferents was by no means as constant (table 2 A) as the pattern of their ipsilateral effects Thus, reverse crossed actions to the ones described above have also been found This is illustrated in Fig 21 A and B In this experiment

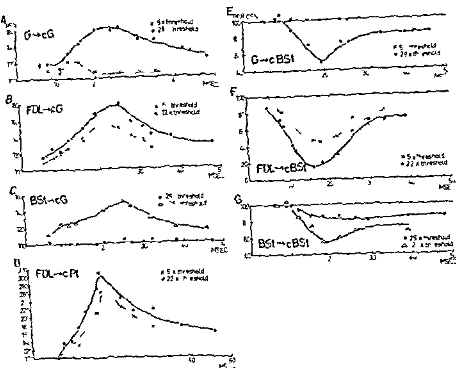


Fig 20 Curves of crossed actions on G (A—C) Pl (D) and BSt (E—G) *monosynaptic* test reflexes evoked by conditioning volleys in the nerves to G (A and E) FDL (B D and F) and BSt (C and G) at stimulus strengths given Comparatively high stimulus strengths for conditioning were required to evoke effects from the BSt nerve (C and G) but an abnormally high strength was also necessary to obtain a maximal group I volley probably because of damage to the nerve Acute spinal (L_4) cat Procedure and conventions as in Fig 1

the contralateral flexors were facilitated and the contralateral extensors inhibited Apparently impulses in high threshold muscle afferents can excite as well as inhibit the same contralateral motor nuclei Of considerable interest is the findings that group II and III volleys do not always reciprocally influence the crossed extensors and flexors This is shown by the two other experiments in Fig 21 one illustrated in C and D and the other in E and F High threshold muscle afferents in one preparation inhibited the extensor G (C) as well as the flexor BSt (D), while in the other preparation both the extensor and the flexor were facilitated (E and F) A further survey of the crossed actions in these three situations will be found in the spinal pattern of Fig 30 31 and 25—29 respectively It is noteworthy that also at a conditioning strength of 5 times threshold

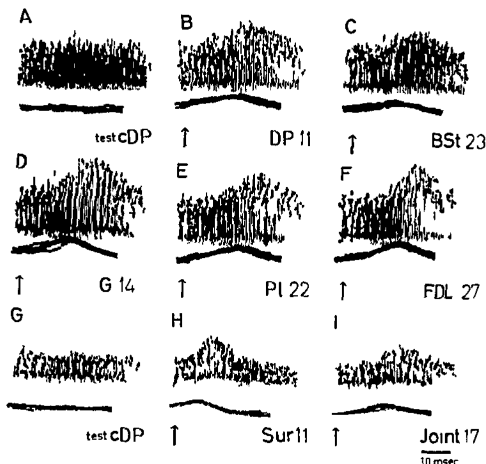


Fig. 19. Crossed actions on DP monosynaptic test reflexes shown unconditioned in A and G, evoked by volleys in the various nerves indicated. Records G—I were taken with lower amplification than records A—F. The same acute spinal (I_2) cat as in Fig. 18. Procedure and conventions as in Fig. 3.

LUNDBERG 1959a). Crossed actions from the FRA in accordance with the scheme of double reciprocal innervation, i.e. excitation to extensor and inhibition to flexor nuclei has also been observed. This is exemplified by the experiment illustrated in Fig. 20, in which volleys in high threshold muscle afferents *give excitation to the contralateral extensor nuclei* G (curves in A—C) and Pl (curves in D) while the contralateral flexor nucleus BSt received inhibition (curves in F—G).

The pattern of crossed actions from high threshold muscle afferents was by no means as constant (table 2A) as the pattern of their ipsilateral effects. Thus *reverse crossed actions to the ones described above have* also been found. This is illustrated in Fig. 21A and B. In this experiment

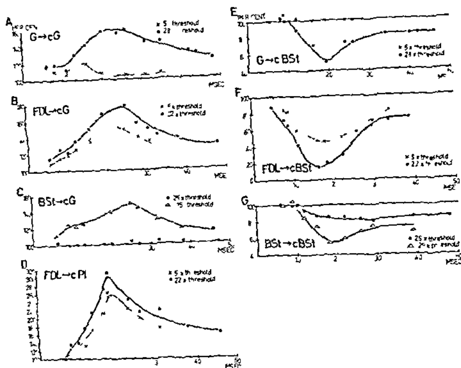


Fig 20 Curves of crossed actions on G (A—C) Pl (D) and BSt (E—G) monosynaptic test reflexes evoked by conditioning volleys in the nerves to G (A and E) FDL (B D and F) and BSt (C and G) at stimulus strengths given Comparatively high stimulus strengths for conditioning were required to evoke effects from the BSt nerve (C and G) but an abnormally high strength was also necessary to obtain a maximal group I volley probably because of damage to the nerve Acute spinal (L₄) cat Procedure and conventions as in Fig 1

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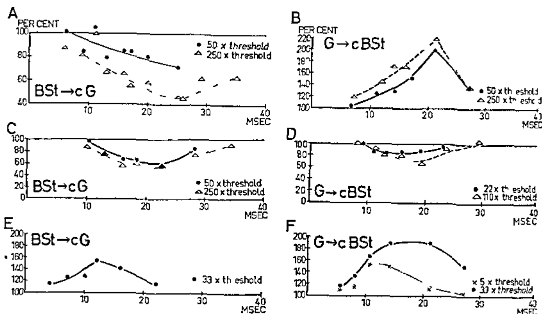


Fig 21 The curves were obtained from three experiments 1) A and B 2) C and D and 3) E and F The curves in A—D were from cats with lesions at obex while the curves in E and F were from a cat with acute section of the cord in L_2 Procedure and conventions as in Fig 1 and 20

actions of opposite modality on the same contralateral nucleus have been found in different preparations

There were also other indications of variability in the crossed actions from the FRA In some preparations there were poor actions to one type of motor nuclei of synergic muscles (extensors or flexors) during the whole course of the experiment, while there were prominent actions to motor nuclei of their antagonists For instance though there was marked crossed facilitation to DP in Fig 18 and 19 and also to BSt there were scarcely any crossed effects to the extensors Q G and Pl in this experiment In other experiments pronounced crossed actions developed during the course of the experiment For example this was the case in the experiment illustrated in Fig 25—28 There the facilitation to the flexors DP (Fig 27) and BSt (Fig 28) could be disclosed very soon after spinal transection and remained fairly unchanged during the following hours whereas facilitation to the extensor nuclei Q (Fig 25), G (Fig 26), and FDL (not illustrated) did not appear until some hours after the transection When crossed actions from high threshold muscle afferents had once been established they generally remained, but marked fluctuations or changes have been observed In some experiments pronounced crossed actions to motor nuclei of synergic muscles disappeared while those to the nuclei of the antagonists did not alter In

Table 2 A

The pattern of crossed actions from high threshold muscle afferents in acute spinal cats Figures within brackets indicate the number of animals with a medial lesion at obex In the remaining animals the cord was transected in Th_{12}—L_1

Action to extensor nuclei	Action to BSt	Number of experiments
+	+	7[1] (4)
+	−	3
−	+	3[2] (1)
−	−	2 (1)
+	0	1
0	+	4
0	−	2 (1)
		22 (7)

[1] In 2 cases also excitation to DP
[2] In 1 case also excitation to DP

Table 2 B

The pattern of crossed actions from high threshold muscle afferents in chronic spinal cats In these animals the cord had been transected in L_1—L_2 2—6 weeks before the experiments Figures within brackets indicate the number of experiments on animals without laminectomy (cf methods)

Action to extensor nuclei	Action to		Number of experiments
	BSt	DP	
+	+	+	2
+	+		5 (3)
+	0	+	1
+	0		1
+	−	+	4
+			3 (1)
			16 (4)

one case there was even a reversal of crossed effects to BSt with a change from inhibition to excitation during the course of the experiment and in another experiment there was initially a crossed pattern of actions in conformity with the crossed extension reflex but after a short interval the modality of crossed actions reversed to both G and BSt and remained so until the cat died one hour later

Table 2 A summarizes the findings in acute spinal cats As mentioned above all four possibilities of variations of crossed actions to extensors and flexors (BSt) have been found but the table shows that the two types with

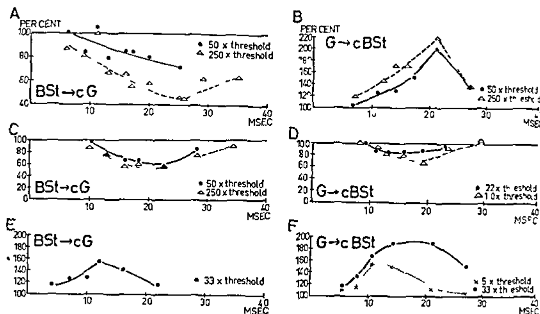


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B Skin afferents and high threshold joint afferents

Impulses in skin and high threshold joint afferents evoke ipsilateral actions resembling those evoked from high threshold muscle afferents This is also true with respect to their crossed actions as exemplified in Fig 19H and I With only a few exceptions it has been found that impulses in skin and joint afferents produce the same modality of actions as impulses in high threshold muscle afferents

Experiments with graded stimulation revealed that no crossed actions from joint afferents could be recorded until the stimulus strength was raised to about 2 5 times threshold for the nerve For these experiments the volley evoked by stimulation of the knee joint nerve was recorded triphasically from the sciatic nerve and the threshold of the nerve could be measured accurately Hence it can be concluded that impulses in the coarse joint afferents do not give rise to any actions in contralateral motoneurones a phenomenon already described for other systems activated by the FRA (OSCARSSON 1957 1958 ECCLES and LUNDBERG 1959 a) Fig 23 shows the effect to the contralateral G monosynaptic test reflex peripherally recorded at increasing strength of stimulation of the posterior knee joint nerve In this experiment the stimulus strengths are expressed in multiples of the threshold for the minimal detectable dorsal horn potential This potential is presumably due to interneuronal activity caused by impulses in joint afferents and cannot be obtained until the stimulus strength is raised above 2 times threshold for the joint nerve Thus the appearance of the facilitatory action at 1 5 times threshold for the dorsal horn potential in A means a stimulus strength > 3 times threshold for the nerve With only a slight rise in stimulus strength the effect increases to a maximal value as shown in C at 3 times threshold for the dorsal horn potential (> 6 times threshold for the nerve) A further increase in the stimulus strength only gives a slight prolongation of the effect as is shown in D at 40 times threshold for the dorsal horn potential (> 80 times threshold for the nerve) The latency for the action has been estimated to 3 msec assuming that the fastest conducting fibres which give effect have a diameter less than 8 μ Sometimes there was a longer central delay for actions evoked by high threshold joint afferents

The curves in Fig 23 were obtained from a chronic spinal cat In these preparations it was observed that the crossed facilitatory actions from the joint nerve were extremely powerful and could often evoke a more prominent effect than a single volley in a muscle or skin nerve Unfortunately the posterior nerve to the knee joint was not dissected in any of the chronic spinal animals with crossed inhibitory actions to BSt thus

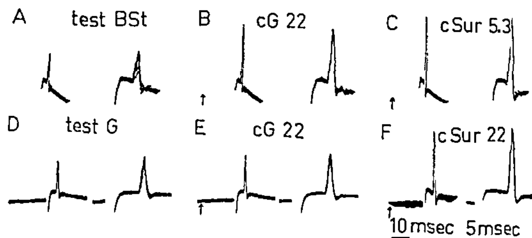


Fig. 23. Monosynaptic reflexes evoked and recorded in the peripheral nerve to BSt (A—C) and G (D—F). Conditioning stimuli were applied to the contralateral G nerve (B and E) and sural nerve (C and F) at times indicated by the arrows. Chronic spinal cat. Procedure and conventions as in Fig. 2. Some records are retouched.

excitation to extensors dominated and the combination with facilitation to flexors was most common. The frequent occurrence of these two combinations in acute spinal cats is of interest because in chronic spinal animals only these two types of patterns were met with (table 2 B).

The common absence of reciprocity of crossed actions by volleys in high threshold muscle afferents was a surprising finding, specially in *chronic spinal animals*, and it may be questioned whether this is an artefact caused by the extensive dissection. In order to reduce the trauma to a minimum, four experiments were made on chronic cats not subjected to laminectomy. The monosynaptic reflex discharges were evoked and recorded peripherally from the muscle nerves as described under methods. In three of these experiments test reflexes to flexors as well as extensors were facilitated (Fig. 22) while facilitation of extensors linked with inhibition of BSt test reflexes was found only in one case (cf. figures within brackets in table 2 B).

On four occasions it was observed in chronic spinal cats that the synergic motor nuclei DP and BSt received opposite actions (cf. table 2 B). The former was facilitated whereas the latter was inhibited. Similar differences (*but in opposite direction*) have also been found for their ipsilateral receptiveness (ECCLES and LUNDBERG 1959 a, PAINTAL 1961, HOLMQVIST and LUNDBERG 1961).

In chronic spinal cats the threshold for crossed actions was often low and quite *prominent crossed actions could often be evoked at conditioning* stimulus strengths slightly supramaximal for group I (cf. Fig. 6).

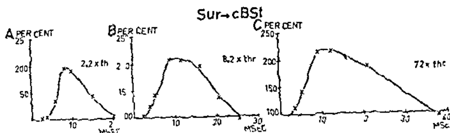


Fig 24 Crossed actions of a conditioning volley in the sural nerve on the BSt monosynaptic reflex recorded peripherally as in Fig 22 The intervals between conditioning and testing stimuli are given on the abscissa Procedure and conventions as in Fig 1

The central delay for the crossed actions evoked by volleys in cutaneous nerves was often much shorter (2 5 msec) than for the actions from the other FRA (3 5—? msec) (cf also Fig 19) This short latency is also apparent in Fig 24 where it has been estimated to be of this order In this figure the actions of a conditioning volley in the sural nerve are shown on the contralateral BSt test reflexes peripherally recorded Graded stimulation revealed that activity in the largest fibres were responsible for a major part of the crossed facilitation (curves in A and B) whereas impulses in thinner fibres only prolonged the facilitatory actions

Discussion

As mentioned in the introduction to this chapter it was found that actions from the flexor reflex afferents (FRA) converge to a number of neuronal systems and that in all cases the receptive field is very wide In the present series on acute spinal animals the various afferent systems comprising the FRA almost invariably evoked the same actions in any given contralateral motor nucleus and also in this case there was convergence from a wide receptive field in particular the convergence of crossed actions from extensor and flexor muscles should be noted In the same preparation the FRA almost invariably gave the same modality of action to all extensor nuclei The only flexor nucleus regularly investigated was BSt When DP occasionally was tested (3 experiments) its receptiveness was the same as that of BSt but in all three cases the effect was excitation to both these flexors It can therefor not be excluded that BSt and DP may have a different receptiveness in ... to ...

... to extensors and flexors (BSt)

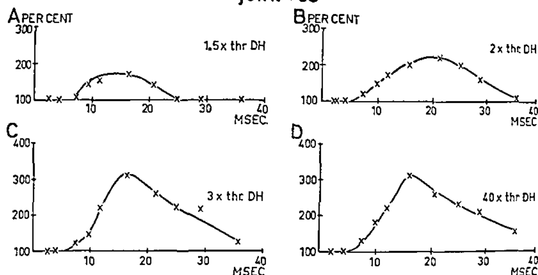


Fig 23 Crossed actions on G monosynaptic reflexes evoked by single conditioning volleys in the posterior knee joint nerve at stimulus strengths expressed in multiples of the minimal observable dorsal horn potential The test reflexes were recorded peripherally (as in Fig 22) The intervals between conditioning and testing stimuli are given on the abscissa Chronic spinal cat Procedure and conventions as in Fig 1

it was not possible to test whether the inhibitory pathways from the joint afferents were equally potent

Crossed effects from the joint nerve were investigated in 9 experiments (7 acute and 2 chronic spinal cats) In 8 experiments these volleys evoked the same actions as high threshold muscle afferents (cf Fig 19 29 and 31)

Cutaneous nerves consist of heterogeneous afferent fibre systems which by adequate activation are known to evoke complex actions to ipsilateral motor nuclei (HAGBARTH 1952) as well as to contralateral motor nuclei (MUGIRIAN 1960) In spite of this a single volley in a skin nerve evokes very similar actions as high threshold muscle and joint afferents to a number of neuronal systems and they have therefore been included among the FRA This investigation has been limited to the crossed actions evoked by stimulation of the sural nerve and the intention has not been to give a complete analysis of the crossed actions of cutaneous origin Usually volleys in the sural nerve gave the same modality of crossed actions as volleys from the other FRA This is illustrated in Fig 19 H Fig 29 C and G, Fig 30 E and Fig 31 C and F Only to 4 motor nuclei in 3 of 17 experiments did volleys in cutaneous nerves and volleys in the other FRA evoke actions of opposite modality

(1906 1910) found that this reflex could be evoked by stimulation of muscle as well as skin nerves and he has actually stated that the afferent nerves which evoke crossed extension include all those which evoke ipsilateral flexion GRAHAM BROWN (1911) first emphasized that crossed flexion also could go together with the ipsilateral flexion reflex (cf also SHERRINGTON 1898) One reason that he observed this more frequently than SHERRINGTON may have been that he studied reflex contractions in ankle muscles whereas SHERRINGTON mainly used knee muscles In a joint study of exceptional reflex reactions GRAHAM BROWN and SHERRINGTON (1912) found that crossed flexion though rare occurred more often at the ankle than at the knee This is interesting since in the present investigation a similar difference has been noted in the crossed receptiveness of BSt and DP in chronic spinal cats To some extent the type of crossed effect obtained may also be due to the time elapsed after spinal transection McCOUCH et al (1935) found that initially (from 20 min to 24 hours after the transection) stimulation of the skin or of a nerve invariably yielded crossed flexion which reflex after a transient period with mixed movements at various contralateral joints changed into crossed extension In the present study one observation in the same direction was made the variable pattern found in acute spinal animals was stabilized in *chronic cats* there was always facilitation to contralateral extensor nuclei although the effect to the contralateral BSt nucleus could be *inhibition or excitation*

Thus there are similarities between the crossed reflex actions studied in earlier investigations and the present one and it might be assumed that essentially *we are dealing with the* same phenomenon There are however also discrepencies in the results as well as the experimental procedure as will be discussed

In many of the earlier *investigations* the muscle tension was recorded in preparations with the gamma loop left intact and crossed actions to gamma motoneurones are *known to exist (HUNT 1951)* but the crossed extensor reflex as well as the crossed flexor reflex have been observed in de afferented muscles (GRAHAM BROWN 1911 1912 1914)

In SHERRINGTON's (1906 1910) experiments reciprocal actions to extensor and flexor nuclei were a *constant feature of the cros*[illegible]

[illegible] ay have been a recurrent Renshaw inhibition caused by discharges in extensor motoneurones on the testing side (cf RENSHAW 1941 ECCLES et al 1954 GRANIT PASCOE and STEG 1957 but also WILSON 1959 WILSON et al 1960) Neither is it known to

1) excitation to extensor and inhibition to flexor (BSt) nuclei
2) excitation to both extensor and flexor nuclei
3) inhibition to extensor and excitation to flexor nuclei
4) inhibition to both extensor and flexor (BSt) nuclei

Combinations with excitation to extensor nuclei 1) and 2) were frequently met with (table 2 A p 41) and were the only variations observed in chronic spinal animals (table 2 B) In the acute spinal preparations the pattern to some extent depended on the interval after transection of the cord but after a few hours the pattern generally remained unchanged although a reversal of the action was occasionally observed There was no difference between high and low acute spinal animals

The fact that volleys evoked by electrical stimulation of a nerve trunk can give effects of opposite sign is in itself not suprising and reversal of reflex actions have been described under a variety of experimental conditions (cf GRAHAM BROWN 1912 PI SUNER and FULTON 1929 CREED et al 1932 JOB 1953) For the interpretation of the present results recent findings on actions by the FRA on ipsilateral motor nuclei should be considered In spinal animals in good condition the FRA give excitatory action to flexor and inhibitory action to extensor motor nuclei Inhibitory action to flexor nuclei is often found and though more exceptionally also excitatory action to extensor nuclei (ECCLES and LUNDBERG 1959 a PAINTAL 1961 HOLMQVIST and LUNDBERG 1959 b 1961) For a number of reasons (HOLMQVIST and LUNDBERG 1961) it has been postulated that the same afferent fibres have two interneuronal pathways with opposite end effects to the same ipsilateral flexor motoneurones The functional organization of the ipsilateral and contralateral effects from the FRA owns so many characteristics in common not least with respect to their supraspinal control (cf next chapter) that it seems reasonable to suggest the same explanation for the crossed pathways i e two interneuronal pathways from the same afferent fibres with opposite end effect on contralateral motoneurones The variability in crossed spinal effects from the FRA often without reciprocity would suggest a more labile relationship between these interneuronal paths than on the ipsilateral side as could perhaps be expected with longer interneuronal chains

As volleys in the FRA evoke ipsilateral actions with a pattern in conformity with that of the flexion reflex (SHERRINGTON 1910) the question arises whether the crossed actions from the FRA also can be correlated with crossed reflex movements The crossed extension reflex with reciprocal actions on extensor and flexor nuclei is well known and SHERRINGTON

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In SHERRINGTON s (1906 1910) experiments reciprocal actions to extensor and flexor nuclei were a constant feature of the crossed extension reflex As described above this was not always found for the crossed effects from the FRA It is not known to which extent the reciprocal inhibition of flexor nuclei in SHERRINGTON s experiments may have been a recurrent Renshaw inhibition caused by discharges in extensor motoneurones on the testing side (cf RENSHAW 1941 ECCLES et al 1954 GRANIT PASCOE and STEG 1957 but also WILSON 1959 WILSON et al 1960) Neither is it known to

1) excitation to extensor and inhibition to flexor (BSt) nuclei
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3) inhibition to extensor and excitation to flexor nuclei
4) inhibition to both extensor and flexor (BSt) nuclei

Combinations with excitation to extensor nuclei 1) and 2) were frequently met with (table 2 A p 41) and were the only variations observed in chronic spinal animals (table 2 B) In the acute spinal preparations the pattern to some extent depended on the interval after transection of the cord but after a few hours the pattern generally remained unchanged although a reversal of the action was occasionally observed There was no difference between high and low acute spinal animals

The fact that volleys evoked by electrical stimulation of a nerve trunk can give effects of opposite sign is in itself not suprising and reversal of reflex actions have been described under a variety of experimental conditions (cf GRAHAM BROWN 1912 PI SUNER and FULTON 1929 CREED et al 1932 JOB 1953) For the interpretation of the present results recent findings on actions by the FRA on ipsilateral motor nuclei should be considered In spinal animals in good condition the FRA give excitatory action to flexor and inhibitory action to extensor motor nuclei Inhibitory action to flexor nuclei is often found and though more exceptionally also excitatory action to extensor nuclei (ECCLES and LUNDBERG 1959 a PAINTAL 1961 HOLMQVIST and LUNDBERG 1959 b 1961) For a number of reasons (HOLMQVIST and LUNDBERG 1961) it has been postulated that the same afferent fibres have two interneuronal pathways with opposite end effects to the same ipsilateral flexor motoneurones The functional organization of the ipsilateral and contralateral effects from the FRA owns so many characteristics in common not least with respect to their supraspinal control (cf next chapter) that it seems reasonable to suggest the same explanation for the crossed pathways i e two interneuronal pathways from the same afferent fibres with opposite end effect on contralateral motoneurones The variability in crossed spinal effects from the FRA often without reciprocity would suggest a more labile relationship between these interneuronal paths than on the ipsilateral side as could perhaps be expected with longer interneuronal chains

As volleys in the FRA evoke ipsilateral actions with a pattern in conformity with that of the flexion reflex (SHERRINGTON 1910) the question arises whether the crossed actions from the FRA also can be correlated with crossed reflex movements The crossed extension reflex with reciprocal actions on extensor and flexor nuclei is well known and SHERRINGTON

Chapter IV

Suprasplnal control of the crossed actions evoked from the flexion reflex afferents

Introduction

Information regarding supraspinal control of the ipsilateral reflex actions by the FRA (flexor reflex afferents) has been derived from a comparison of these actions in the decerebrate and in the spinal states. SHERRINGTON and SOWTON (1915) demonstrated with accurate methods that the flexor reflex was easier to elicit in the spinal than in the decerebrate animal. They assumed that this was due to higher excitability of flexor motoneurones in the spinal than in the decerebrate state. FULTON (1926) suggested that in the decerebrate state inhibition of the interneurones transmitting these actions may be another explanation and later investigations have shown that there is such a tonic inhibition of interneurones of the flexion reflex pathways in the decerebrate state (JOB 1953, ECCLES and LUNDBERG 1958, 1959 b, KUNO and PERL 1960).

This tonic inhibition of interneurones of ipsilateral reflex arcs can act independently of the cerebellum and the vestibular nuclei, hence it can be differentiated from the tonic control responsible for decerebrate rigidity (HOLMQVIST and LUNDBERG 1959 a). The centers responsible for the tonic inhibitory control of flexor reflex pathways are located medially in the brain stem and their descending pathways are located in the dorsal part of the lateral funiculi and from each side a bilateral effect is exerted (HOLMQVIST and LUNDBERG 1959 a). Experiments with lesions at different levels in the lower reticular formation have revealed a differential control of inhibitory pathways to extensor motoneurones and of excitatory to flexor motoneurones. A lesion in the lower pons releases inhibitory pathways to ipsilateral extensor motor nuclei whereas a more caudal lesion is necessary for release of the excitatory pathways to flexor motoneurones. In addition it has been found that a low pontine lesion also releases an inhibitory pathway from the FRA to ipsilateral flexor motoneurones (HOLMQVIST and LUNDBERG 1959 b, 1961). A similar organization has now been established for the supraspinal control of the crossed actions from the FRA. The supraspinal control of the decerebrate preparation will be described in section A. Section B deals with the effects of a low pontine lesion

which extent the nociceptive systems have been activated in the different investigations and whether impulses in these fibres more constantly can give rise to the reciprocal actions of the crossed extensor reflex

In the present investigation the latencies of the crossed effects evoked from the FRA were relatively short SHERRINGTON (1910, cf also CREED et al 1932) found very much longer latencies for the crossed extension reflex and repetitive stimulation was usually necessary to evoke the reflex In later investigations by ECCLES and GRANIT (1929) and FORBES and CATTELL (1924) considerably shorter latencies were found It is important to re member that most of these experiments were made on decerebrate cats and the results are therefor not directly comparable with effects observed in spinal cats Crossed reflex pathways are suppressed in the decerebrate state (cf next chapter), and one reason for the long latencies may have been that temporal facilitation was necessary to overcome the suppression from supraspinal centres However, in chronic spinal 'deafferented' cats MATTHES and RUCH (1933) have found a crossed reflex contraction in soleus evoked by a single stimulus after a latency of a similar order as that now found for the crossed facilitation

Summary

In spinal cats single volleys in skin, high threshold joint and high thresh old muscle afferents evoke similar actions in any given contralateral motor nucleus The following minimal central delays were found for vol leys in high threshold muscle afferents 3—4 msec in high threshold joint afferents, 5 msec, and skin afferents 2 5 msec The duration of the action was about 25 msec

In acute spinal animals motor nuclei of synergic muscles (extensors or flexors) usually received the same modality of action However, the pat tern of actions varied in different preparations and all four possible varia tions of actions were met with

1) facilitation to extensor and inhibition to flexor (BSt) nuclei
2) facilitation to both extensor and flexor nuclei
3) inhibition to extensor and facilitation to flexor nuclei
4) inhibition to both extensor and flexor (BSt) nuclei

The first two types were common in acute spinal animals and in chronic spinal cats the *only types observed*

In chronic spinal cats DP received excitation regardless whether BSt was excited or inhibited

The variability in the pattern of crossed actions has been discussed in connection with previous investigations on crossed reflexes

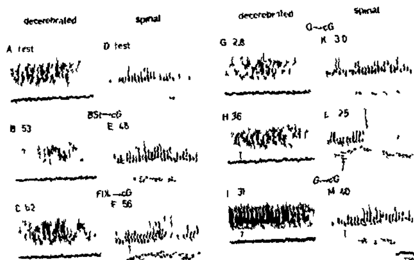


Fig 26 Crossed actions on the G monosynaptic reflexes of single conditioning volleys in the various nerves indicated in the decerebrate and spinal states The *same experiment* as in Fig. 25 27 and 28 Procedure and conventions as in Fig 3 and 25 Some records are retouched

contralateral Q nerve with a Ia volley (record I) and a *just maximal* group I volley (record K) Neither were there any crossed *effects* by group I volleys after section of the cord (record N, cf chapter II) For control ipsilateral group I effects were sometimes recorded in the decerebrate state and potent Ia reciprocal inhibition was found (cf Fig 28 B)

by

br [illegible] are given *in Fig* 25—28 Each figure represents the actions to one motor nucleus *and the records* are arranged in pairs, with the left obtained before and *the right after* transection of the cord in L

Fig 25 illustrates the effects on the contralateral Q test reflexes, shown unconditioned in A and F *Before spinal* section volleys in high threshold muscle afferents from G FDL BSt and Q (*records* B—D L and M) did not give any crossed actions but *after section* of the cord corresponding volleys gave large facilitatory *effects* (records F—H P and R) For the Q nerve this phenomenon *is shown at different* stimulus strengths and for all stimulus strengths *up to 36 times threshold* (I—M), there was the same lack of actions in the decerebrate state in spite *of* a good segmental

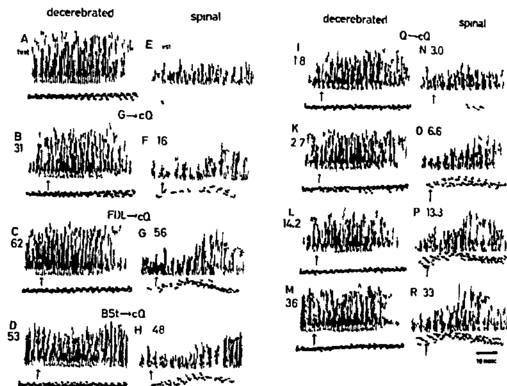


Fig 25 The crossed actions on the Q monosynaptic reflexes of single conditioning volleys in the various nerves indicated Records A—D and I—M were obtained after intercollicular section and E—H and N—R after section of the cord in L_1 The same experiment as in Fig 26—28 Procedure and conventions as in Fig 3 Some records are retouched

Results

A Comparison of crossed actions in the decerebrate and spinal states

In these experiments on decerebrate cats (intercollicular section) the effects of conditioning volleys on contralateral monosynaptic reflexes were studied in the decerebrate state and compared with the effects subsequently obtained after section of the cord in L_1 or after a medial lesion at obex (cf p 35 chapter III) both giving a release to the spinal state

In the decerebrate state there was the same absence of crossed group I actions on monosynaptic test reflexes as has been found in the spinal state (cf chapter II) When the group I volley displayed the separation in the Ia and Ib components it was also ascertained that neither the Ia nor the Ib volley evoked any crossed effect This is exemplified in Fig 25 where the monosynaptic reflex from Q was conditioned from the

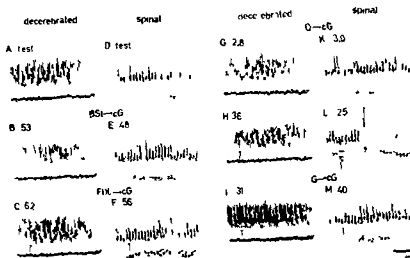


Fig 26 Crossed actions on the G monosynaptic reflexes of single conditioning volleys in the various nerves indicated in the decerebrate and spinal states The same experiment as in Fig 25 27 and 28 Procedure and conventions as in Fig 3 and 25 Some records are retouched

contralateral Q nerve with a Ia volley (record I) and a just maximal group I volley (record K) Neither were there any crossed effects by group I volleys after section of the cord (record N, cf chapter II) For control ipsilateral group I effects were sometimes recorded in the decerebrate state and potent Ia reciprocal inhibition was found (cf Fig 28 B)

A representative survey of the difference between crossed actions evoked by a conditioning volley in high threshold muscle afferents in the decerebrate and spinal states are given in Fig 25—28 Each figure represents the actions to one motor nucleus and the records are arranged in pairs with the left obtained before and the right after transection of the cord in L_2

Fig 25 illustrates the effects on the contralateral Q test reflexes shown unconditioned in A and E. *Before* spinal section volleys in high threshold muscle afferents from G FDL BSt and Q (records B—D L and M) did not give any crossed actions but after *section of the cord* corresponding volleys gave large facilitatory effects (records F—H, P and R) For the Q nerve this phenomenon is *shown at* different stimulus strengths and for all stimulus strengths up to 36 times threshold (I—M), there was the same lack of actions in the *decerebrate state* in spite of a good segmental

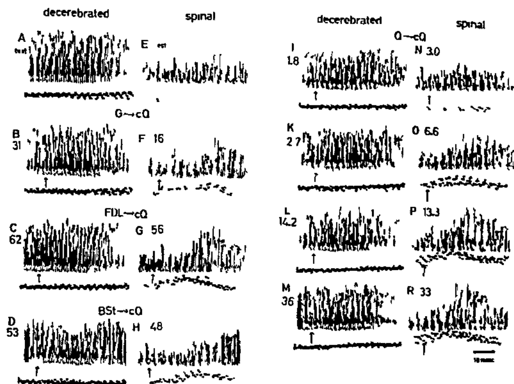


Fig. 25. The crossed actions on the Q monosynaptic reflexes of single conditioning volleys in the various nerves indicated. Record A—D and I—M were obtained after intercollicular section and E—H and N—R after section of the cord in L_2. The same experiment as in Fig. 26—28. Procedure and conventions as in Fig. 3.
Some records are retouched.

Results

A. Comparison of crossed actions in the decerebrate and spinal states

In these experiments on decerebrate cats (intercollicular section) the effects of conditioning volleys on contralateral monosynaptic reflexes were studied in the decerebrate state and compared with the effects subsequently obtained after section of the cord in L_2 or after a medial lesion at obex (cf. p. 35, chapter III) both giving a release to the spinal state.

In the decerebrate state there was the same absence of crossed group I actions on monosynaptic test reflexes as has been found in the spinal state (cf. chapter II). When the group I volley displayed the separation in the Ia and Ib components it was also ascertained that neither the Ia nor the Ib volley evoked any crossed effect. This is exemplified in Fig. 25 where the monosynaptic reflex from Q was conditioned from the

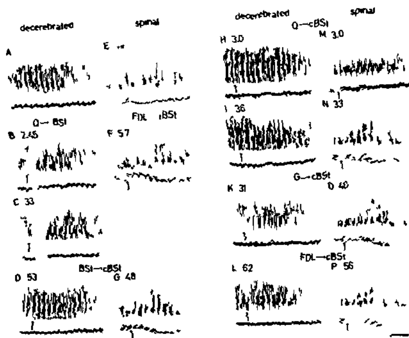


Fig 28 Crossed actions on the BSt monosynaptic *reflexes of* single conditioning volleys in the various nerves indicated in the *decerebrate and spinal states* The same experiment as in Fig 25—27 Procedure and conventions as in Fig 3 and 25

other hand in Fig 28 there is in the *decerebrate state* no crossed effect to BSt from BSt (D) and *FDL* (*L*) but crossed inhibition from Q (I) and probably also a trace from G (K) Nevertheless after section of the cord the facilitatory effects from Q and G (N and O) resembled those from the other nerves (G and P) *This inhibition* from contralateral Q (I) is of special interest since it was *found that* a corresponding volley in the ipsilateral Q also inhibited the BSt test *reflex* *This is* shown in record C, where the first inhibitory phase was caused by *impulses* in the I a afferents (cf record B) whereas the *additional later inhibitory* phase was due to activity in high threshold afferents Such an *inhibitory* action from FRA to ipsilateral flexor nuclei is occasionally *met with in decerebrate* preparations (cf ECCLES and LUNDBERG 1959 b Fig 2 HOLMQVIST and LUNDBERG 1959 a Fig 3 and 5 HOLMQVIST and LUNDBERG 1961) The bilateral inhibitory effects from Q have been *interpreted* as due to a partial release from

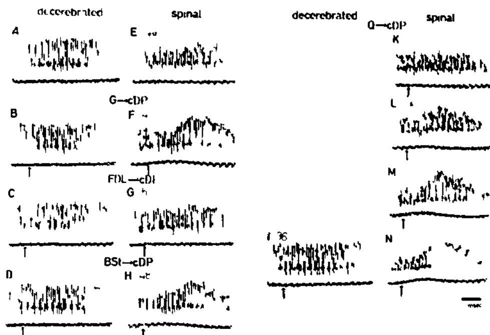


Fig 27 Crossed actions on the DP test reflexes of single conditioning volleys in the various nerves indicated in the decerebrate and spinal states The same experiment as in Fig 25 26 and 28 Procdure and conventions as in Fig 3 and 25

condition of the cord indicated by the low threshold for the crossed effect after spinal section Thus already a group II volley at 6 6 times threshold evoked significant facilitation (O) which increased when group III fibres were activated (P and R) It is suggested that the suppression of actions in B—D L and M is due to a tonic inhibition from supraspinal centers on the pathways mediating the actions from the FRA

The same features were also found to the other contralateral extensor nuclei investigated G (Fig 26) and FDL (not illustrated) In no instance was there in this series any sign of crossed actions by impulses in high threshold muscle afferents in the decerebrate condition (Fig 26 B C H and I) although pronounced crossed facilitation to contralateral G (Fig 26 L F L and M) and to contralateral FDL was found in the spinal state

Effects on crossed pathways to flexor nuclei are illustrated in Fig 27 and 28 In Fig 27 there is similarly no crossed effect to DP by volleys in high threshold muscle afferents in the decerebrate state (B D and I) but release of facilitation in the spinal state (F—H and K—N) On the

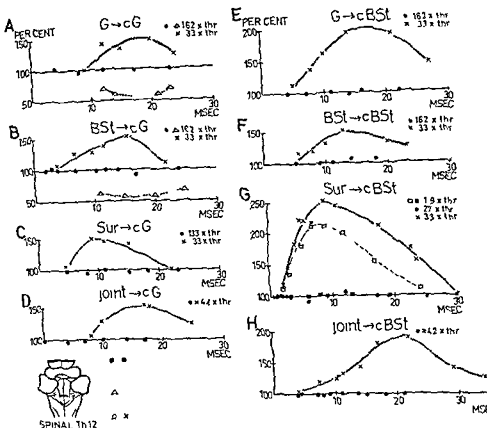


Fig 29 Crossed actions of single conditioning volleys in the various nerves indicated on the monosynaptic reflexes from G (A—D) and from BSt (E—H) in the decerebrate state (■●) after a pontine lesion (△) as indicated in the schematic drawing and after spinal section in Th₁₂ (□×) Procedure and conventions as in Fig 1

was measured and was found to be high (The other experiment was not analysed in this respect) It is possible that the ipsilateral cutaneous effects which escape the control in the decerebrate state should not be included among the actions of the FRA, but other explanations cannot be excluded

The decerebrate cat illustrated in Fig 29 was cerebellectomized (cf also Fig 30 and 31) whereas in the experiments illustrated in Fig 25—28 the cerebellum was left intact No difference has been observed between these two types of preparations (cf HOLMQVIST and LUNDBERG 1959 a)

the otherwise complete blockage in the decerebrate state of pathways mediating inhibitory actions from FRA (cf section B in this chapter)

It should be noted that the sizes of the unconditioned test reflexes before and after transection of the cord do not reflect corresponding changes in alpha excitability because double volleys were often used to evoke test reflexes (cf methods) After section of the cord the excitability was usually increased in flexor and decreased in extensor motoneurones In the present investigation it was attempted to use test reflexes of equal size before and after section of the cord as for the DP test in Fig 27 (A and E) but sometimes this was not possible In Fig 25 26 and 28 the test reflexes are smaller in the spinal than in the decerebrate state The reason is that in these experiments double stimuli could not be used for evoking the test reflexes after section of the cord This may be connected with the often marked spontaneous fluctuations found in the spinal state (presumably because of the release of reflex arcs from supraspinal control) Therefore it was difficult to keep the first test volley subliminal particularly when a conditioning volley gave facilitation but sometimes also for the unconditioned test reflexes In several experiments it was, however, controlled that conditioning volleys in the FRA had no action in the decerebrate state even when smaller test reflexes were used

Also Fig 29 is from a decerebrate preparation, which shows not only the suppression of crossed spinal actions evoked by volleys in high threshold muscle afferents on contralateral G (A and B) and BSt (E and F) test reflexes but also of the crossed actions from the other FRA, namely of cutaneous (C and G) and articular (D and H) origin The curves in G show that neither at weak (■) nor at strong (●) stimulus strengths were there any crossed effects from the sural nerve to BSt in the decerebrate state, in spite of quite pronounced facilitatory actions (□ ×) after section of the cord This is interesting because even in decerebrate preparations with very effective control of actions to other pathways activated by the FRA there is usually *from the sural nerve an early ipsilateral facilitatory* action both to flexors and extensors which is evoked by impulses in large cutaneous afferents (HOLMQVIST and LUNDBERG 1961) In the experiment illustrated in Fig 29 there was also in the decerebrate state such ipsilateral facilitatory action to BSt from the largest fibres in the sural nerve and exclusively from these *fibres In 11 of 13 decerebrate cats there was com*plete suppression of crossed effects from the sural nerve In 7 of these cats, there were ipsilateral facilitatory actions in the decerebrate state from the sural nerve as has been described for the preparation in Fig 29 (In the remaining 4 experiments the ipsilateral effect was not controlled) However, *in 2* experiments there was a slight crossed facilitatory action from the sural nerve, in one case to both BSt and G in the other only to G In the former experiment the threshold at which the effect appeared

itantly released inhibitory actions to the contralateral G (curves in A and B) and BSt (curves in C—E) This was found for actions by all FRA high threshold muscle afferents (A—D) skin afferents (E) and high threshold joint afferents (cf Fig 31 G) After a more caudal lesion there was also release of facilitatory actions by the FRA It was found that a medial lesion in the region of obex (×) was as effective as a complete transection of the cord and henceforth this preparation will be regarded as spinal (cf p 35)

In Fig 30 the crossed spinal pattern was inhibition to extensors and facilitation to flexors which is one of the spinal patterns described in chapter III In a similar experiment partly illustrated in Fig 29 a low pontine lesion (△) was also followed by a general inhibition which is shown only for contralateral G (curves in A and B) In the spinal state there was in this case a reversal to general facilitation of contralateral extensor and flexor test reflexes (×) In the experiment illustrated in Fig 31 on the other hand the general inhibition revealed by the pontine lesion (△) remained on the contralateral side *after* a lesion at obex (×) and transection of the cord (not illustrated)

In low pontine preparations a comparison of ipsilateral and contralateral inhibitory effects from the FRA has revealed that the effects were always more marked ipsilaterally On the ipsilateral side this lesion gives an almost complete release of the inhibitory pathways to extensor motoneurones and concomitantly a release of inhibitory paths to flexor motoneurones from the FRA (HOLMQVIST and LUNDBERG 1959b 1961) The ipsilateral inhibition to flexor motoneurones is often weak but in the experiments illustrated in Fig 30 and 31 there was on the ipsilateral side almost complete inhibition of the BSt test reflexes from FDL (Fig 30 G and Fig 31 I) On the contralateral side the test reflexes were seldom reduced by more than 50 per cent (Fig 30 A—E Fig 31 A—G) These crossed inhibitory effects after a low pontine lesion could only in one experiment be evoked by volleys in group II muscle afferents (conditioning stimulus strength 5 times threshold) in 9 other experiments group III volleys were required Probably the absence of *crossed group II* effects after the low pontine lesion in most of the experiments has no special significance because absence of group II effects is also common for the ipsilateral actions after a low pontine lesion (HOLMQVIST and LUNDBERG 1961)

Both in Fig 30 and 31 the crossed inhibitory actions were larger after a low pontine lesion than in the spinal state Possibly this may be due to spinal shock but another factor may be release of excitatory paths (cf chapter III) By intracellular recording from motoneurones it has been

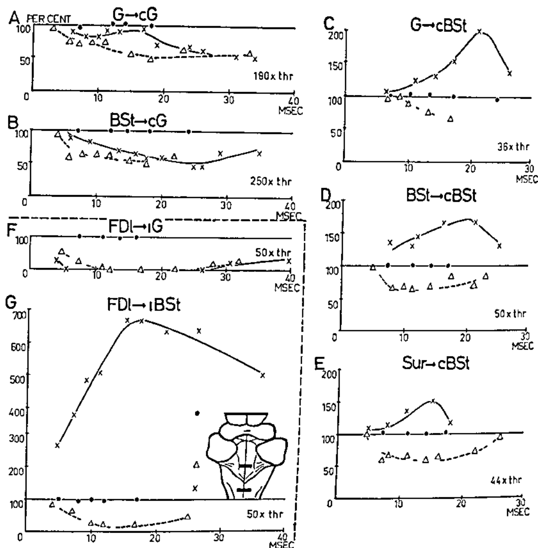


Fig 30 Contralateral (A—E) and ipsilateral (F and G) actions on the G (A B and F) and on the BSt (C—E and G) test reflexes evoked by single conditioning volleys in the various nerves indicated after lesions given in the schematic drawing Procedure and conventions as in Fig 1 and 29

B *Effects of lesions in the brain stem on crossed actions*

In the present investigation the actions from the FRA to motor nuclei of both sides were compared at successive more caudal lesions in the brain stem It was found that a low pontine lesion at the level indicated in the schematic drawing of Fig 30, which released inhibitory actions (△) to the ipsilateral G (curve in F) and BSt (curve in G) concom

Discussion

It has been established that in the decerebrate state single volleys in the FRA usually do not evoke any actions on contralateral monosynaptic test reflexes but after a medial lesion at obex or transection of the cord there is a release of crossed spinal actions such as were described in chapter III The suppression of crossed actions from the FRA in the decerebrate state much resembles what has been found for their ipsilateral actions (ECCLES and LUNDBERG 1959 b) and it seems reasonable to suggest that also interneurones of the paths from the FRA to contralateral motoneurones are tonically inhibited from the brain stem in the decerebrate state in a similar fashion as has been concluded for their ipsilateral actions No crossed group I effects were observed in the decerebrate state in this series but since there were no effects after section of the cord this finding leaves the question open whether there is a similar supraspinal control of crossed group I pathways as has been found for the ipsilateral Ib pathways (ECCLES and LUNDBERG 1959 b)

In decerebrate cats SHERRINGTON (1910) found that spinal transection gave an immediate fall in threshold and volume of the ipsilateral flexion reflex but a rise in threshold and fall in supraliminal response of the crossed extensor reflex As suggested by SHERRINGTON (1910) this rise in threshold of the crossed reflex may be due to a fall in excitability of alpha motoneurones to extensors following the spinal transection Another possibility is that crossed reflex excitation of gamma motoneurones (HUNT 1951 GRANIT 1955) may be different in the decerebrate and spinal states This possibility deserves special attention since the decerebrate rigidity is maintained by the gamma loop (cf GRANIT 1955)

Further evidence of parallelism in supraspinal control of ipsilateral and contralateral reflex pathways from the FRA has been given in the experiments on decerebrate cerebellectomized animals with a low pontine lesion This lesion which releases inhibitory reflex pathways from the FRA to ipsilateral extensor as well as flexor motoneurones (HOLMQVIST and LUNDBERG 1959 b 1961) also gives release of inhibitory paths from the FRA to contralateral extensor and flexor motor nuclei whereas a more caudal lesion is necessary to release excitatory pathways to both sides

For the ipsilateral reflex actions from the FRA it has been postulated that supraspinal centres differentially can control actions to flexor and extensor motoneurones and also that they can select between an excitatory and an inhibitory channel to flexor motoneurones (HOLMQVIST and LUNDBERG 1961) On similar grounds it is suggested that supraspinal centres differentially may govern FRA pathways to contralateral motoneurones

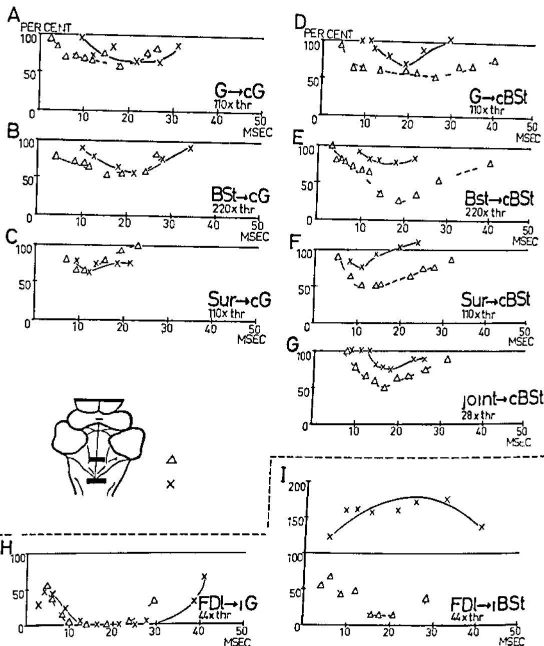


Fig 31 Contralateral (A—G) and ipsilateral (H and I) actions on the G (A—C and H) and on the BSt (D—G and I) monosynaptic test reflexes evoked by single conditioning volleys in the various nerves indicated after lesions given in the schematic drawing Procedure and conventions as in Fig 1 and 29

confirmed that impulses in the FRA may evoke excitatory or inhibitory synaptic actions in motoneurones of the same contralateral nucleus and also that individual motoneurones may receive both these crossed actions (HOLMQVIST and LUNDBERG unpublished)

Comments and Summary

This investigation deals with the crossed spinal reflex actions to alpha motoneurones from various myelinated afferents in the hindlimb of the cat

It has not been possible to distinguish between crossed effects evoked by impulses in Ia and Ib afferents but to a certain extent the experimental series on crossed group I effects must be considered as introductory to further experiments with intracellular technique Recording of synaptic potentials in individual motoneurones presumably gives a better chance to utilize the slow and fast components of the group I volley At the same time the present findings are of importance for the future analysis It has now been revealed that repetitive stimulation is required to evoke clear cut crossed group I action and a quantitative frame for actions expected in various motor nuclei has been erected Of particular interest for further analysis of the crossed group I actions is

1) The source of the crossed excitation between extensors
2) The absence of crossed inhibitory actions between extensors
3) The difference in crossed receptiveness of DP and BSt as well as the differencies in crossed actions evoked by volleys from these muscles
4) The possibility of crossed presynaptic inhibition Inhibitory group I effects may be due to presynaptic inhibition (Frank and Fuortes 1957 Eccles Eccles and Magni 1960 Eccles 1961) and such effects will be difficult to disclose without intracellular recording

The investigation of actions evoked by volleys in the FRA (group II and III muscle skin and high threshold joint afferents) perhaps permits more definite suggestions Contralateral motoneurones provide a new neuronal system showing convergence of effects from group II and III muscle afferents from high threshold joint afferents and skin afferents and the concept of the unity of actions evoked by the FRA has thereby been strengthened (cf Eccles and Lundberg 1959a Lundberg 1959 Holmqvist et al 1960)

In view of the relative constancy of the crossed extensor reflex and the well established concept of double reciprocal innervation it was surpris

This suggestion may help to explain the puzzling findings in spinal animals, in which FRA volleys under apparently the same conditions but in different preparations can evoke opposite crossed effects in the same motor nucleus Possibly the supraspinal centres may select suitable inhibitory or excitatory channels to contralateral motoneurones and it is not necessary to assume that reciprocal innervation of flexor and extensor nuclei is maintained in this selection

It should, however, be noted that the experiments with pontine lesions have revealed only that the centres controlling inhibitory and excitatory pathways have a different localization in the brain stem The degree of functional freedom between these centres cannot be decided by experiments of the present type It is neither possible to make any suggestions about the functional linkages between these controlling systems located at the same brain stem level Even if there are great resemblances in the release from supraspinal control of ipsilateral and crossed reflex arcs after lesions at various brain stem levels (cf Fig 28) it is nevertheless possible that the supraspinal centres may control these reflex arcs independently

Summary

It has been established that the pathways of the crossed actions from the flexor reflex afferents (FRA) are under a similar supraspinal control as has been found for the corresponding ipsilateral paths

In decerebrate cats single volleys in skin, high threshold joint and muscle afferents have no actions on contralateral monosynaptic test reflexes but after a medial lesion at obex or transection of the cord in the upper lumbar region a release of crossed spinal actions from these afferent systems occurs It is postulated that in the decerebrate state there is a supraspinal tonic inhibitory control of the interneurones mediating crossed actions from the FRA

There is evidence of a separate control of pathways mediating the facilitatory and inhibitory crossed actions because a medial lesion in the low pontine reticular formation releases only inhibitory actions from the FRA to contralateral flexor nuclei as well as to extensor nuclei A more caudal lesion is necessary to release the crossed facilitatory actions

Comments and Summary

This investigation deals with the crossed spinal reflex actions to alpha motoneurones from various myelinated afferents in the hindlimb of the cat. It has not been possible to distinguish between crossed effects evoked by impulses in Ia and Ib afferents but to a certain extent the experimental series on crossed group I effects must be considered as introductory to further experiments with intracellular technique. Recording of synaptic potentials in individual motoneurones presumably gives a better chance to utilize the slow and fast components of the group I volley. At the same time the present findings are of importance for the future analysis. It has now been revealed that repetitive stimulation is required to evoke clear cut crossed group I action and a quantitative frame for actions expected in various motor nuclei has been erected. Of particular interest for further analysis of the crossed group I actions is

1) The source of the crossed excitation between extensors
2) The absence of crossed inhibitory actions between extensors
3) The difference in crossed receptiveness of DP and BSt as well as the differencies in crossed actions evoked by volleys from these muscles
4) The possibility of crossed presynaptic inhibition. Inhibitory group I effects may be due to presynaptic inhibition (FRANK and FUORTES 1957, ECCLES, ECCLES and MAGNI 1960, ECCLES 1961) and such effects will be difficult to disclose without intracellular recording

The investigation of actions evoked by volleys in the FRA (group II and III muscle, skin and high threshold joint afferents) perhaps permits more definite suggestions. Contralateral motoneurones provide a new neuronal system showing convergence of effects from group II and III muscle afferents, from high threshold joint afferents and skin afferents and the concept of the unity of actions evoked by the FRA has thereby been strengthened (cf. ECCLES and LUNDBERG 1959a, LUNDBERG 1959, HOLMQVIST et al. 1960).

In view of the relative constancy of the crossed extensor reflex and the well established concept of double reciprocal innervation it was surpris

ing that volleys in the FRA could evoke either excitation or inhibition in flexor and extensor nuclei and that the combination with facilitation to both extensor and flexor nuclei was particularly common In this case preliminary experiments with intracellular recording have revealed inhibitory and excitatory synaptic potentials in flexor as well as extensor motor neurones (HOLMQVIST and LUNDBERG, unpublished) The possibility remains that these non reciprocal crossed actions are evoked by afferents with different receptive function but another suggestion seems more likely i e that the same afferent fibres may have two alternative paths to a given contralateral motor nucleus This has been postulated to account for the dual actions from the FRA to flexor nuclei on the ipsilateral side (ECCLES and LUNDBERG 1959 a, HOLMQVIST and LUNDBERG 1959 b, 1961) and the present suggestion for the contralateral connections is given by analogy On the ipsilateral side the reciprocal flexor reflex actions from the FRA are very dominant in spinal preparations in good condition but apparently there is a more labile relationship between the corresponding crossed actions This may be due merely to longer internuncial chains in the crossed paths but it is possible that differential actions on the contralateral side are more often required in the various reflex movements of the animals

The experiments on the supraspinal control of crossed actions were concerned exclusively with the actions from the FRA and have shown a parallelism with the control of ipsilateral reflex arcs (ECCLES and LUNDBERG 1959 b, HOLMQVIST and LUNDBERG 1959 b, 1961) There is likewise suppression of action in the decerebrate state and release of inhibition to both contralateral extensor and flexor motor nuclei after a low pontine lesion Thus the low pontine preparation is characterized by general inhibition with loss of reciprocal and double reciprocal innervation A more caudal lesion in the medulla is necessary to release crossed excitatory actions from the FRA

It is true that some of the crossed spinal effects disclosed in this investigation were weak but this does not necessarily mean that their functional significance is negligible since some reflex arcs may receive facilitation from supraspinal centres It has recently been shown that interneurones of ipsilateral reflex arcs are facilitated by impulses in the pyramidal tract (LUNDBERG and VOORHOEVE 1961)

The work was originally suggested by Professor Anders Lundberg I wish to express my sincere gratitude for his stimulating advice and valuable help Thanks are due to Mr E Eide Miss Karin Ebbesson and Miss Solveig Jeppsson for much appreciated technical assistance

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I. INTRODUCTION

The purpose of the prensent investigation was to study the relation between the cell density and the spontaneous spike and slow wave activity in different cortical layers. Previous investigations on the relationship between these two different types of activity and their correlation to different cortical structures have been limited to a few selected cortical layers. The aim of the present investigations was to study the distribution of spike and slow wave activities in different layers throughout the entire cortical depth. The claustro-insular area, i.e. *area insularis agranularis anterior dorsalis* — Ai (according to the terminology of Rose 1928) in the rabbit has been chosen as the object of this study, since its different cortical layers with varying cell density are relatively well differentiated. The fact that this area including the claustral layer is thicker than other parts of the cerebral cortex in the rabbit offers the possibility of recording activity from several separate points at different depths, a circumstance of significance for the statistical treatment of the material.

The spontaneous activity at different cortical depths has been recorded with an extracellular microelectrode, and the distribution of the spike potentials and the slow wave activity has been studied in different cortical layers, of which the relative cell density has been estimated on the basis of histological studies. The influence of varying depths of anesthesia on the spatial distribution of the different types of spontaneous activity has also been studied. The material obtained has been treated statistically.

Some of the results have been described in a preliminary report (Valleala 1960).

of the electrode tip in relation to the size of the cell (see e g HAAPANEN et al 1958) When the tip of the electrode penetrates the cell structure so called injury potentials may appear Before the appearance of injury potentials i e when the electrode is being pushed towards the cell spike potentials are often recorded within a limited zone The polarity and configuration of these spike potentials change essentially before the microelectrode has achieved a localization at which it evokes injury potentials These spike potentials may be called *proximity potentials* LI (1955) has described the general features of these proximity potentials and their fluctuations as a function of the position of the electrode tip in relation to the cell When the electrode enters the active zone negative potentials are recorded which may be followed by a small positive potential When the electrode is moved further a sudden change of polarity often occurs and the potentials become positive negative The initial positive component is larger and grows further when the electrode is moved closer to the cell At a certain point positive spikes of several tenths of a millivolt occur When a momentary negative DC shift appears these positive spikes may develop into high frequency injury potentials which then cease abruptly

MOUNTCASTLE et al (1957) studied the depth dimension of the area from which proximity potentials could be obtained when evoked by different types of stimulation In their studies made with slightly larger microelectrodes (tip diameter 2—4 μ) the proximity potentials were seldom found to be positive (for dimension see FRANK 1955) The only change which they obtained when the electrode was moved towards the cell was a diminution of the amplitude of negative spike potentials Generally, injury potentials were not obtained which led these workers to assume that in their experiments the electrode often by passed the neuron The depth dimension of the area from which proximity potential were obtained was found to be about 100 μ

LI and JASPER (1953) reported that the size of the spike potentials — in their investigations negative — is affected not only by proximity to but also by the volume of the perikaryon of the active cell in the vicinity of the electrode tip Thus, they recorded their maximum spike potentials from the layer of the large pyramidal cells

II SURVEY OF MICROELECTRODE STUDIES WITH SPECIAL REFERENCE TO THE SPONTANEOUS CORTICAL ACTIVITY

RENSHAW, FORBES and MORISON (1940) in their classical microelectrode study on the electrical activity of isocortex and hippocampus discussed the spatial accuracy of the extracellular microelectrode technique. In the hippocampus they recorded low amplitude negative spike potentials which they assumed to represent activity in single neurons or small functionally homogeneous neuron groups. The origin of these spike potentials with a duration of about 1 msec was assumed to be the perikaryon rich layer of the pyramidal cells in the Ammon's horn or the vicinity of this layer.

WOLDRING and DIRKEN (1950) studied the spontaneous cortical activity in anesthetized rabbits. According to them the activity consisted of three components: 1) a heterogeneous background activity, 2) slow waves which were generally irregular in shape and mode of occurence and which on rare occasions appeared in spindle shaped trains, 3) spike activity which was affected by anesthetics. Curare had no effect on the spike activity provided that ventilation was insured.

Successful attempts later on were made to refine and standardize the microelectrode method by using glass micropipettes filled with 3 M KCl solution (LING and GERARD 1949). The tips of the micropipettes are less than 1 μ in diameter and the resistance generally varies between 1 and 50 megohms. Most publications deal with the response to electrical and different types of natural stimulation but they also present information on the nature of spontaneous activity (BAUMGARTEN & JUNG 1952, AMASSIAN 1953, BUSER & ALBE-FESSARD 1953, TASAKI *et al* 1954, LI & JASPER 1953, PHILLIPS 1956 a, MOUNTCASTLE *et al* 1957). All workers report the appearance of membrane potential changes when the electrode passes through the cortex. Some investigators have mainly been interested in these membrane potentials (LI 1955, 1959, PHILLIPS 1956 a and b).

Even a small electrode obviously causes damage to the cell and the damage is naturally more severe the larger the diameter

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attention to the more differentiated picture of the cortical activity obtained by an intracortical microelectrode than that obtained by surface electrode recording. A considerable advantage of the microelectrode technique is that an electrode of this type makes close contact with the structural elements. The spontaneous electrical activity recorded with a microelectrode can be considered as a wave spectrum in which the duration of the individual waves gradually changes from short to long. In the short duration periphery of the spectrum it is possible to distinguish the spike potentials which are fairly sharply divided from the other part of the spectrum.

According to the above mentioned workers these types of activity can be characterised as follows.

The *spike potentials* follow the "all or none" law and show a precise spatial localisation in zones of a few microns to tens of microns in diameter in the depth dimension of the cortex. The rise and fall of spike potential frequency may be regarded as signs of increase and decrease in excitability. The spike activity disappears in anoxia and in deep anesthesia.

The *slow potential* activity is usually more heterogeneous and not as well defined as the spike activity. The slow potentials are graded and not of "all or none" type. Their spatial distribution in the cerebral cortex is not as distinct as that of the spike potentials and their pattern often shows considerable variations in recordings from a single point. The variation often increases with the duration of the waves. In deep anesthesia and anoxia there is a lowering of the frequency and decrease in amplitude.

Li & Jasper (1953) considered the relationship between spike activity and slow activity to be complex and variable. According to them some units tend to discharge with slow waves, others do not. Those units which discharged with waves had a tendency to fire in bursts. Verzeano & Calma (1954) reported that the relationship between the spikes and the slow waves was completely fortuitous in the alert animal but that these types of activity were synchronized when the animal was under deep Nembutal anesthesia characterized by spindle activity. These investigators used steel electrodes with a tip diameter ranging from 5 to 10 μ and recorded negative spikes with low

These workers further stated that *the zone of the proximity* potentials is not markedly greater than the size of the neurosome. They assumed that the fall in the gradient of the electric *field is fairly sharp and that it is difficult to map the limit of* this field with high resistance electrodes because of the high noise level. They also emphasized the difficulty in recording po*tentials from small cells and fibres* with an electrode with such properties. The records obtained will thus represent a selected group of the spontaneously active cells.

Tower and Amassian (1958) reported that when a large spike amplitude was recorded, the area from which proximity potentials could be obtained is also large and concluded that a large volume of the nerve element is involved.

The potential fluctuations of the electrocorticogram recorded with gross electrodes, are of long time duration. The possible counterpart to the EEG slow activity in the intracortical microelectrode records has consequently attracted great attention. According to Renshaw *et al.* (1940) it is not probable that the slow waves of the EEG are composed of spike potentials. Most workers refer to the long time factor of the dendritic potentials (Chang 1951, 1952, 1955) which according to Clare and Bishop are graded (1955 a and b).

Distinct differences have been stressed between the axone and *the soma response on one hand and the dendritic response on* the other. Tasaki *et al.* (1954) for instance stated the potential duration in the axon to be somewhat below 1 msec., in the neuronal soma *slightly over 1 msec. and in the dendrites* 15—20 msec.

Renshaw *et al.* (1940) using a pair of electrodes with an interelectrode distance of *105—300 μ were able to record potentials* of 30—150 msec. duration which showed a tendency to be localised to certain cortical depths. Mountcastle *et al.* (1957) recorded a slow wave type *the spatial extent of which varied be*tween 20 μ and 70 μ (average 32 μ). This type of activity was found in all layers of the cortex. Their preparation was under a light Penthotal anesthesia.

The relationship between spike activity and slow activity has been the object of many studies. Li and Jasper (1953) paid

The claustrum which lies latero-basally, is separated from the medially situated putamen by the external capsule. Laterally, the claustrum is bounded by the extreme capsule which varies greatly in size and compactness in different species. According to RAE (1954) who reviewed the literature on the claustrum and its connections silver-impregnated sections show an intricate fibro-cellular reticulum. The cell bodies are scattered without apparent order in the mesh of this network. There is no precise information concerning the possible synapses between the reticulum and the claustral cells.

ROSE (1928) described the cytoarchitecture of the insula in many animals and man. ROSE, like BRODMANN, includes claustrum in the insula. Following this line of approach he included in the insula the pre-piriform area belonging to the claustro-cortex. The pre-piriform area is well developed in macrosmatic mammals. It is structurally related to the rhinencephalon and separated from the neo-pallial part of the insula by the rhinal sulcus. According to BRODMANN (1909) the appearance of the insula in mammals is constant and in area the largest uniform part of the cerebral cortex. It is best developed in man but is, however, overshadowed by the other neopallial structures.

B. The claustro-insular area in the rabbit

ROSE's description (1928, 1931) and terminology of the claustro-insular area has been followed in this paper. The insular area which according to ROSE is agranular in lower mammals, is clearly divided in the rabbit into a dorsal granular and ventral agranular part bordering on the rhinal sulcus. The extent of the insular area is easy to estimate owing to the poor folding of the cerebral cortex of the rabbit. BRODMANN (1909) stated the ventral *width* of the insular area to be a third of the height of the hemisphere and its length to be *more than half of the* hemisphere. In the rabbit the claustrum is topographically closely connected with the cerebral cortex covering it since the extreme capsule is only partly visible but is distinguishable by the special character of its structural elements.

amplitude According to them a train of negative spikes was always *followed by the positive slow wave* The investigators concluded that the slow positive wave represents summated positive after potentials In a later investigation (VERZEANO *et al* 1955) the various results concerning the ratio between slow activity and spike activity obtained by different workers were discussed The divergences were attributed, at least in part, to differences in the preparation and in the recording technique As mentioned above, MOUNTCASTLE *et al* (1957) described a *type of spatial grouped spontaneous* slow wave activity which however, was not accompanied by spike activity It is to be noted that reports of concurrent spike activity and slow activity generally refer to spike potentials of negative polarity and relatively small amplitude

III SURVEY OF ANATOMICAL STUDIES OF THE CLAUSTRO INSULAR AREA

A General

In the present investigation the term claustro insular area' refers to the claustrum and the insular section of the cerebral cortex *covering this nucleus In man and primates the ventral* part of the claustrum — fragmented into individual nuclei — partly extends into the temporal lobe, *i e* outside the traditional insula BROCKHAUS (1940) used the concept claustro cortex to denote the part of the cerebral cortex which covers the claustrum

The claustro insular area constitutes a regional entity the ontogenetic and histological homology of which has been the subject of considerable controversy The greatest divergence of opinion *has concerned the question of* whether the claustrum originates as a fragment of the deepest layer of the cerebral cortex enclosing it the insular area or whether its genesis is independent of the cerebral cortex The former view was *upheld* by *e g* BRODMAN (1909) ARIENS KAPPERS (1921) ROSE (1928 1931) and the latter by *e g* CAJAL (1902) VON LANDAU (1923) VON ECONOMO and KOSKINAS (1925) BROCKHAUS (1940) FILIMONOFF (1947) MACCHI (1951)

The claustrum, which lies latero basally, is separated from the medially situated putamen by the external capsule Laterally, the claustrum is bounded by the extreme capsule which varies greatly in size and compactness in different species According to RAE (1954), who reviewed the literature on the claustrum and its connections silver impregnated sections show an intricate fibro-cellular reticulum The cell bodies are scattered without apparent order in the mesh of this network There is no precise information concerning the possible synapses between the reticulum and the claustral cells

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Fig. 1. Lateral view of the rabbit's brain. B = Bulbus olfactorius. Rh = rhinal sulcus. The dark area above the rhinal sulcus indicates the position of Area insularis agranularis (after Rose, 1928).

The present investigation concerns the agranular part of the insular area only *(Area insularis agranularis anterior dorsalis)* (Fig. 1). The characteristics of the layers of the insular area in the rabbit can be described as follows (Fig. 2).

The zonal lamina (I) is fairly broad. Layers II and III are contiguous containing small or medium sized pyramidal cells which are often grouped in dense clusters. The layer corresponding to the granular layer (IV) is absent. The ganglionic cell layer (V) is broad and divided into three sublayers a, b and c. Va and Vc are visible as rather light zones in a Nissl stained preparation. Vb is broad and contains large radially arranged pyramidal cells. The pyramidal cells of the underlying multiform layer (VI) are small and narrow. The claustrum is very closely connected with the sixth layer (with which layer VII is also frequently assimilated). The cells of the claustrum are of medium size and large stellate and fusiform. In some places cells resembling the pyramidal cells are seen.

According to Zunino (1909) and Winkler & Potter (1911) the fiber structure of the insular cortex is very scanty in the rabbit. Rose (1928, 1931) reported that the rostral part of the insular area shows no fibres of the extreme capsule. Le Gros Clark & Meyer (1947) by removing the olfactory bulb and tracing degenerated fibres showed that the olfactory tract also terminates

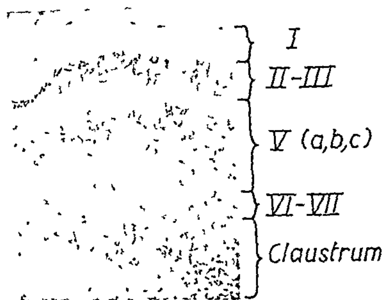


Fig. 2. Microphotograph of a transverse section through *Area insularis agranularis* showing the different horizontal layers (nomenclature after Rose). Thionine ×24

in the prepiriform area which could be regarded as an olfacto-projective area. ALLISON (1950) using a corresponding method showed a connection between the prepiriform area and the agranular insular area. This finding in the rabbit in a way supported the general hypothesis put forward by ROSE (1928) that the agranular insular area is closely connected with the rhinencephalon and that it participates in the olfactory function. No direct connections have been found between the area insularis anterior dorsalis (ai) and the hypothalamus (LUNDBERG 1960).

IV. METHODS

Animal preparation. The investigations were carried out on rabbits having a mean weight of 2.6 kg (range 2.2—3.5 kg). Before operation 30 mg/kg of Nembutal was given intravenously

and Lidocain (1 % solution) was injected around the operative field Tracheotomy was followed by evisceration of the right eye The orbital skull bone was removed on the right side and the anterior part of the rhinal sulcus was exposed Bleeding was carefully stopped by ligation or cauterisation

Approximately 5 × 5 mm of the dura was removed in the immediate vicinity of the rhinal sulcus, 7 mm occipitally from the frontal end of the tissue A small celluloid plate with a hole (1 × 2 mm) in its centre was placed on the exposed part of the brain with the hole over the place where the dura was removed The arterial pulsations did not seriously interfere with the recordings The movements of the brain caused by respiration, however, was considerable at times These movements were damped by placing a thin layer of cotton wool between the celluloid plate and the edges of the bone Microscopic control was made in order to check that the pial circulation was intact

The head of the animal was rigidly fixed in a holder The holder was fitted with a ball and socket joint by which the head could be tilted laterally so that the surface of the brain under examination lay as nearly horizontal as possible This permitted the electrode to be manipulated in a vertical direction in order to be inserted perpendicularly to the brain surface The operative field was covered with paraffin kept at a temperature of ca 38° C

Recording Glass capillaries filled with 2 7 M KCl were used as recording electrodes (resistance 1—20 megohms) The micro electrode was inserted vertically into the brain through the hole in the celluloid window with the aid of a Zeiss micromanipulator The insertion was carried out under visual control through a stereomicroscope The indifferent lead was a gross Ag AgCl electrode placed in the subcutaneous tissue

The potentials were recorded with the aid of a cathode follower (HAMMARÉN & OTTOSON 1954) and an AC coupled (time constant 200 msec) Nagard oscilloscope amplifier A 2 channel analyzer and a loudspeaker were connected The time integral of the slow potentials was recorded by one channel with a coupling time constant of 500 msec The other channel gave a count of all positive spikes exceeding 1 mV In all experiments the ampli

fication was kept constant at 1 mV/inch The noise level varied 100 200 microvolts Some potentials were photographed with a Grass camera

The electrode was inserted into the claustro insular area 1 mm dorsally from the rhinal sulcus and about 7 mm occipitally from the frontal end of the rhinal sulcus This area corresponds to that described by ROSE at the anterior dorsal agranular insular area (Fig 1 and 2) The microelectrode was inserted in steps of 50 μ and recordings were made at each 50 μ step for a period of 15 sec If the various conditions of the preparation during the electrode insertion were found to be constant, the insertion was extended to a depth not over 3200 μ, which depth corresponds to the maximal value of the thickness of the claustro insular area measured from histological preparations Insertions which did not extend a depth of 2500 μ were not included in the material The activity recorded at each step was assumed to represent the average activity at that step including a distance of 25 μ above and below the recording point

In some cases anesthesia introduced before the operation (30 mg/kg of Nembutal intravenously) was deepened in the final stage of the operation (after about 1 ½—2 hours) with 30 mg/kg of Nembutal intraperitoneally, or 5 to 20 mg/kg of Nembutal intravenously Lidocain was injected now and then into the skin around the wound

The recordings were divided into two groups according to the depth of anesthesia

The first group comprised recordings obtained at a stage in the experiment at which the animal was under light anesthesia (LA group on an average 5 mg/kg two hours before recording) In this group curare was given as a rule in small doses (0 2—0 6 mg/kg) and the animal was maintained on artificial respiration

The other group comprised recordings obtained under deep anesthesia (DA group on an average 20 mg/kg two hours before and on an average 13 mg/kg one hour before recording) In order to obtain a constant level of anesthesia throughout the experiment in the DA group additional Nembutal doses (2 5—5 0 mg/kg) were given also during the recording phase (approximately every 15—30 min c f FORBES *et al* 1956) In order to avoid overdosage

the heart rate and respiration were checked regularly in the course of the experiment

The electrode resistance was checked during the experiment. If a distinct change was noted, the recordings were rejected. The recordings were also rejected when the preparation showed signs of impaired circulation in the pial vessels or if abnormal cortical activity appeared (e.g. spreading depression, LEAO 1944 a and b) and in the case of abrupt change in the general condition of the animal during the experiment. As a result, the number of succesful complete insertions was relatively small in relation to the great number of experiments carried out.

The insertion depths indicated by the micrometer were divided into zones, 100 μ in width. In order to allow the recording point to represent the middle of each zone, a depth of 25 μ was taken as the upper limit of the first zone. Thus, the first zone covered 25—125 μ below the surface of the brain. In the following, 125 μ refers to the zone 25—125 μ etc. All recording data were referred to these zones. The mean of the integrator values (i.e. the voltage time integrals obtained during 15 sec.) recorded from two successive electrode locations e.g. at the depth of 50 μ and 100 μ, was regarded to be representative for the slow activity within one zone, in this case the zone 25—125 μ (marked as 125 in the diagrams). The spike potential activity was observed on the oscilloscope and the positive spike potentials exceeding 1 mV were counted with the aid of an electronic counter. The probability for positive spike potentials to appear was calculated.

Since relatively few positive spike potentials exceeding the 1 mV amplitude level appeared the total insertion depth was divided in larger zones each zone including 800 μ. The number of recordings (15 sec. duration) including large positive spike potentials from each 800 μ zone was expressed in per cent of all recordings made from this zone.

Distinct injury potentials were not taken into account.

In order to obtain a more detailed picture, the electrode was in some cases moved in 10 μ steps. The material so obtained was not treated statistically since the long duration of the experiment made a comparison difficult between the data obtained at the surface and those obtained from the deep layers of the cortex.

In two experiments the electrode was moved in 100 μ steps These recordings were also excluded from the material treated statistically

Histological preparation The rabbit was chosen because this animal is characterized by few folds in the cerebral cortex, which helps in the assessment of the histological architecture (Bok 1929)

Histological sections were actually taken from each animal which had been used for electrophysiological analysis However, it was found that the electrode insertions often caused structural injuries which made a systematic cell analysis difficult Therefore, histological material was also collected from intact rabbits which had not been subjected to the electrophysiological experiment The weights of these rabbits — 28 in all — were of the same order of magnitude as of those of the rabbits which were used in the physiological experiments, their mean weight being 2 7 kg (range 2 3—2 8 kg) The sections were taken from the region corresponding to that from which the recordings were made

The brains were perfused via the carotid artery first with Ringer's solution and then with 10 per cent formalin The right hemisphere was detached after perfusion and kept in 10 per cent formalin solution for 1—2 days The sections were cut from a block detached perpendicularly to the surface of brain The sections 40 μ thick were prepared as follows rinsing in running water freezing and cutting staining (0 1 per cent Thionine distilled water 95 per cent alcohol absolute alcohol, xylene Canadabalsam)

One section from each of the 28 animals was selected for the histological analysis The perikaryon projections to be seen in the section are of course frequently smaller than the entire maximal section area of the cell The cell projections from each section were drawn on millimeter paper with the aid of a carbon arc projector Glial cells were *not drawn* The magnification was 500 x The tracing was made directly from a rectangular area of the same depth as the claustro insular area and of a width of 200 μ perpendicularly oriented to the surface of the brain and located 1 mm dorsally from the rhinal sulcus — corresponding to the area of the microelectrode insertions The rec

the heart rate and respiration were checked regularly in the course of the experiment

The electrode resistance was checked during the experiment If a distinct change was noted, the recordings were rejected The recordings were also rejected when the preparation showed signs of impaired circulation in the pial vessels or if abnormal cortical activity appeared (e g spreading depression Leão 1944 a and b) and in the case of abrupt change in the general condition of the animal during the experiment As a result the number of succesful complete insertions was relatively small in relation to the great number of experiments carried out

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Distinct injury potentials were not taken into account

In order to obtain a more detailed picture, the electrode was in some cases moved in 10 μ steps The material so obtained was not treated statistically since the long duration of the experiment made a comparison difficult between the data obtained at the surface and those obtained from the deep layers of the cortex

greater is the projection Although the size of the projection also depends on the position of the cell in three dimensional space, it gives a relative measure of the size of the neuron The intention of this histological analysis was not to measure absolute quantities but to describe the relative amount of cells of varying magnitudes at different depths

The line sampling was always performed in the same direction, *i e* parallel to the tangent to the surface of the cerebral cortex This procedure was regarded as sufficiently accurate since pyramidal cells within the claustro-insular area are arranged uniformly with the apex of the pyramid directed radially to the surface of the brain If this were not so, one projection of a pyramidal cell calculated as above would give a different area value from a projection of the same pyramidal cell in another position The only exception consists of certain pyramidal cells in layers II—III of the insular area and in the claustrum proper In the present work however, the number of irregularly situated pyramidal cells was so small that the error in area determination was hardly significant

The term number of cells mentioned in the treatment of the results refers to the number of perikaryon projections

Statistical methods The statistical treatment of the material comprises 32 zones These zones represent individuals whose morphological and physiological characteristics are compared The mutual independence of the individuals thus selected is fairly great owing to the special nature of the recording technique employed The microelectrode is capable of selectively recording the activity from a small tissue volume Thanks to the recording technique each zone exhibited electric properties which made it possible to distinguish the zones The possibility of treating the statistical qualities of the electrophysiological material within the above zonal distribution is also based on this individuality of the zones This would not be possible if larger electrodes had been used

Standard methods of statistical analysis were applied to the data thus obtained using the following formulae

If the arithmetic mean of a variable x which represents a certain characteristic in the sample of n individuals is denoted

tangles were divided into 100 μ zones beginning from a depth of 25 μ. The total number of cell projections exceeding a section surface of 80 μ^2 was counted for each zone (100 × 200 × 40 μ). Projections of cells *appearing to be of borderline area (80 μ^2)* were estimated by line sampling using a modification of the method reported by UOTILA and KANNAS (1952). When a calculation of this kind is used it is *necessary to take into consideration* also the parts of cell projections that are formed by the horizontal and vertical boundaries of the zone. These parts were excluded when they were below the lower limit.

Cells with a projection area smaller than 80 μ were not taken into consideration in the calculation in order to achieve a relevant correlation between morphological and functional data, since it was not possible with the aid of a microelectrode to record succesfully from the smallest perikarya. Satisfactory resting potentials have been successfully measured in the spinal interneurons only in cells with an estimated diameter larger than 10—20 μ (HAAPANEN *et al.* 1958). The electrodes used in the present investigation were however generally slightly larger than those used by HAAPANEN *et al.* If the area (80 μ^2) chosen is the lower limit in the present investigation is assumed to be circular, its diameter is about 10 μ. It is possible that transient injury potentials were often caused by *small cells but no distinct* injury potentials were taken into account in the present investigation.

The *largest perikarya* projections were separated from the population measured by means of the above mentioned line sampling method. Projections larger than about 200 μ^2 were included in this separate group. If this area is assumed to be circular, its diameter is about 16 μ. Since the large projections were relatively few in number a rougher zonal division was used as in the treatment of positive spike potentials. The rectangle 200 μ in width described above was divided into zones of 800 μ. Projections larger than 200 μ^2 were counted as the per cent of all projections within the zone.

The method chosen for *cytological* analysis is thus concerned with two dimensional cell projections and not with cell volumes. In general terms it can said that the larger the perikaryon the

$$r = \frac{s_{xy}}{s_x s_y},$$

where

$$s_{xy} = \frac{1}{n-1} \sum_{i=1}^{n} (x_i - \bar{x})(y_i - \bar{y})$$

and s_x and s_y represent the standard deviations of x and y, respectively.

To find out at which level of significance the correlation between x and y differs from the 0 the t test was again used using the formula

$$t = \frac{r}{\sqrt{1 - r^2}} \sqrt{n-2},$$

number of degrees of freedom $f = n - 2$.

The coefficient of partial correlation between variables 1 and 2 taking variable 3 as constant was calculated from the formula

$$r_{12 \cdot 3} = \frac{r_{12} - r_{13}\, r_{23}}{\sqrt{(1 - r_{13}^2)(1 - r_{23}^2)}}$$

When drawing conclusions with the aid of the t values the risk P was obtained from statistical tables. P indicates the level of significance, i.e. the probability that the difference observed is due to chance. As has been often customary the following expressions were used to interpret the different levels of significance:

Not significant (—)	$0.05 < P$
Significant (s)	$0.01 < P < 0.05$
Highly significant (h.s.)	$0.001 < P < 0.01$
Very highly significant (v.h.s.)	$P < 0.001$

by $\bar{y}$, the standard deviation of x, viz s_x, was obtained from the

$$s_x^2 = \frac{1}{n-1} \sum_{i=1}^{n} (x_i - \bar{x})^2$$

and the standard error of the mean from the formula

$$s\{\bar{x}\} = \frac{s_x}{\sqrt{n}}$$

To estimate the level of significance of the difference of two means, $\bar{x}$ and $\bar{y}$, the following calculation was made

$$t = \frac{\bar{x} - \bar{y}}{s\sqrt{\frac{1}{n_x} + \frac{1}{n_y}}}$$

in which

$$s^2 = \frac{(n_x - 1) s_x^2 + (n_y - 1) s_y^2}{n_x + n_y - 2}$$

and the distribution of which is Student's distribution with the following number of degrees of freedom $f = n_x + n_y - 2$

The standard error of the percentage p was calculated from the formula

$$s\{p\} = \sqrt{\frac{p(100 - p)}{n}}$$

The significance of the difference between two percentages was also estimated with the t test using the following calculation

$$t = \frac{p_1 - p_2}{s\{p_1 - p_2\}},$$

where

$$s^2\{p_1 - p_2\} = s^2\{p_1\} + s^2\{p_2\}$$

and the distribution of which is the Student distribution with the following number of degrees of freedom

$$f = n_1 + n_2 - 2$$

The correlation coefficient between two variables x and y was calculated from the formula

Table 2 Number of cells (column 2) per unit zone (see text) at different depths (mean standard deviation and standard error) 28 histological preparations from the claustro-insular area.

Zone (μ)	Mean $\bar{x}$	St. D s_x	St. E $s\{\bar{x}\}$
125	—	—	—
225	—	—	—
325	1.5	2.3	0.43
425	6.9	5.6	1.1
525	8.3	5.2	0.98
625	8.7	4.9	0.93
725	6.2	3.7	0.69
825	5.9	3.9	0.75
925	8.2	4.5	0.84
1025	7.9	3.5	0.66
1125	9.1	3.3	0.62
1225	9.2	3.5	0.65
1325	8.9	4.6	0.86
1425	7.5	3.5	0.66
1525	6.1	3.7	0.70
1625	7.0	3.0	0.57
1725	6.8	3.4	0.65
1825	7.5	3.4	0.64
1925	7.2	3.1	0.59
2025	8.6	3.6	0.67
2125	7.9	4.1	0.77
2225	7.5	4.1	0.79
2325	7.3	3.8	0.73
2425	6.2	2.7	0.54
2525	6.6	3.3	0.69
2625	6.7	2.3	0.51
2725	6.3	3.5	0.85
2825	4.4	2.3	0.65
2925	5.4	2.4	0.84
3025	7.3	4.8	1.8
3125	7.6	3.2	1.4
3225	3.0	6.6	4.6

V RESULTS

A Density of claustro-insular cells

The microphotograph of a Thionine stained claustro-insular preparation in Fig 2 gives a general idea of the periodic variation of cell density at different cortical depths As mentioned above, the fluctuation of the cell density called for the designation of various horizontal layers as seen in Fig 2

The histological material in the present work was obtained from 28 animals, from each of which only one section was taken These animals were not used in the physiological experiments (see above)

Although the purpose of the present work was not to localise the recording points within each 'classical" layer, it was none the less desirable to obtain a general idea of the depths of the various horizontal layers in the present material A small magnification of the histological structure of the claustro insular area stained by the Nissl method (Fig 2) shows four principal perikaryon layers II—III, V b, VI—VII and the claustrum Among these layers, VI—VII is the narrowest and least distinct

In all preparations, the depths were measured from the surface of the cerebral cortex to the superficial boundary of the four principal perikaryon layers The total depth of the claustro insular area was also measured The first boundary *i e* between

Table 1 Depths in μ of the borderlines between different horizontal layers in the claustro insular area from 28 histological preparations (mean, standard deviation and standard error)

Borderline / Depth	Mean $\bar{x}$	St D s_x	St E $s_{(\bar{x})}$
I/II—III	320 μ	40 μ	8 2 μ
Va/Vb	810 μ	100 μ	18 9 μ
Vc/VI—VII	1710 μ	200 μ	38 2 μ
VI—VII/claustrum	2000 μ	250 μ	47 3 μ
claustrum/white matter	2740 μ	350 μ	66 5 μ

Table 2 Number of cells (column 2) per unit zone (see text) at different depths (mean, standard deviation and standard error) 28 histological preparations from the claustro-insular area.

Zone (μ)	Mean $\bar{x}$	St. D s_x	St. E $s\{\bar{x}\}$
125	—	—	—
225	—	—	—
325	1.5	2.3	0.43
425	6.9	5.6	1.1
525	8.3	5.2	0.98
625	8.7	4.9	0.93
725	6.2	3.7	0.69
825	5.0	3.9	0.75
925	8.2	4.5	0.84
1025	7.9	3.5	0.66
1125	9.1	3.3	0.62
1225	9.2	3.5	0.65
1325	8.9	4.6	0.86
1425	7.5	3.5	0.66
1525	6.1	3.7	0.70
1625	7.0	3.0	0.57
1725	6.8	3.4	0.65
1825	7.5	3.4	0.64
1925	7.2	3.1	0.59
2025	8.6	3.6	0.67
2125	7.0	4.1	0.77
2225	7.5	4.1	0.79
2325	7.3	3.8	0.73
2425	6.2	2.7	0.54
2525	6.6	3.3	0.69
2625	6.7	2.3	0.51
2725	6.3	3.5	0.85
2825	4.4	2.3	0.65
2925	5.4	2.4	0.84
3025	7.3	4.8	1.8
3125	7.6	3.2	1.4
3225	3.0	6.6	4.6

the layers I and II—III) is the most distinct since the dividing line between the perikaryon poor and perikaryon rich layers is very sharp. The claustrum is also fairly sharply demarcated from the white substance. The most difficult boundary to define was that between layer VI—VII *and the claustrum*. Table 1 shows the depths of the borderlines of the four layers mentioned above (means, standard deviations and standard errors) obtained from above mentioned measurements. According to Table 1 the boundary between the true insular cortex and the claustral layer lies at the depth of $2000 \pm 47.5\ \mu$. The depth of the inner borderline of the claustro-insular area in this material is $2740 \pm 66.5\ \mu$.

Table 2 shows the cell density at the different depths (means, standard deviations and standard errors) measured on the same 28 histological preparations. The cell density concept in this case *refers to the number of perikaryon projections larger than 80* μ^2 per *unit zone* $(100 \times 200 \times 40\ \mu)$.

The diagram in Fig. 3 shows the number of cells per unit zone (vertical axis) at different depths (horizontal axis, zero point represented by a depth of 25 μ, see above). The means are calculated from 28 preparations. As seen in the diagram the surface layer does not include any cell projection larger than 80 μ^2 (*c.f.* layer I in Fig. 2). There are four peaks which are localised around the depths of 625 μ (*i.e.* the zone 575—625 μ), 1225 μ, 2025 μ and 3025 μ, which peaks obviously correspond to the above mentioned horizontal perikaryon layers II—III, V b, VI—VII and *claustrum* (see Fig. 2).

Table 3 shows the statistical significance of the four peaks in Fig. 3. The statistical *estimation* was made in such way that in each peak the zone including the largest mean cell number was compared *with the intervening zones representing the smallest* mean cell number. An evaluation of the significance of the differences between the means of these two types of zones was made. Table 3 shows that all four peaks are statistically significant.

Large cells. Since the positive spike potentials of large amplitude may be regarded as signs of activity *from large perikarya*, *the largest perikarya* of the population in the histological material were separated *for special analysis*. Thus all perikaryon projections

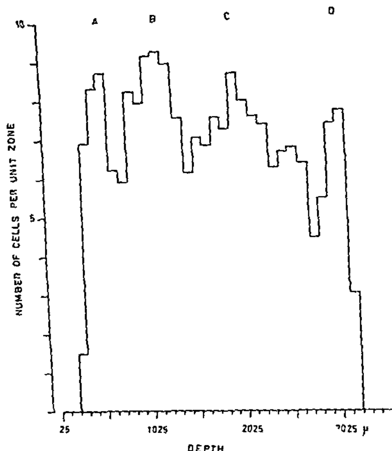


Fig 3 Number of cells per unit zone (vertical axis) at different depths (horizontal axis) Zero point is represented by a depth of 25 μ. A B C and D represent main peaks

with an area of more than 200 μ^2 were specially treated These cells were relatively few in number and were therefore counted in four 800 μ zones which were obtained by combining 100 μ zones in four groups Thus the cell density in this special case is represented by the number of perikaryon projections larger than 200 μ^2 per 8 unit zones (800 × 200 × 40 μ)

The diagram in Fig 4 shows at different depths the pro

Table 3. Evaluation of the statistical significance of the principal peaks in diagram 3 showing number of cells per unit zone at different depths

Peak in diagram 3	Zones (μ)	Difference of means	Number of degrees of freedom f	t value	Stat. sign
A	{ 625 & 825	8 7—5 9	54	2.37	s.
B	{1225 & 825	9 2—5 9	54	3 38	h s.
	1225 & 1525	9 2—6 1	54	3 21	h s.
C	{2025 & 1525	8 6—6 1	54	2 59	s.
	2025 & 2425	8 6—6.2	52	2 81	h s
D	{3125 & 2825	7.6—4 4	15	2 34	s

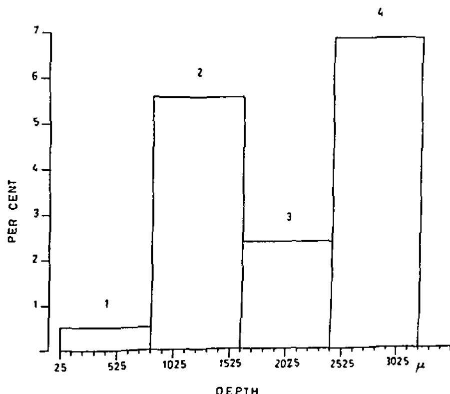


Fig 4 Number of large cells in per cent of all cells counted in 8 unit zones (vertical axis) at different depths (horizontal axis)

portion of cell projections larger than 200 μ This proportion of large cells is expressed in terms of the per cent of all cells counted in 8 unit zones at different depths

Table 4 shows the proportion of large cells in per cent of all cells counted in zones 800 μ thick, and also the standard deviations and standard errors

The calculated density of large cells in zones of this type — although arbitrary — illustrates the variations of cell density in the claustro insular area in such a way that zone 1 (Fig 4) includes layer II—III (Fig 2), zone 2 (Fig 4) includes layer V b (Fig 2) and zone 4 the claustrum Zone 3 (Fig 4) is more heterogeneous including both the layers VI—VII and a part of the claustrum (Fig 2)

As seen in Table 4 and Fig 4 large cells aggregate in zone 2 (layer V b) and zone 4 (claustrum) Table 5 shows that the percentage of number of large cells within these zones differ statistically from the corresponding figure for segment 3

Table 4 The proportion of large cells in per cent of all cells per 8 unit zones 28 histological preparations

8 unit zones No	Zones (μ)	Number of large cells	Per cent	St. E s(p)
1	from 125 to 825	5	0 50	0 21
2	from 925 to 1625	69	5 6	0 58
3	from 1725 to 2425	39	2 4	0 38
4	from 2525 to 3225	40	6 9	1 05

Table 5 Evaluation of the statistical significance of the differences between layers 4 and 3 between layers 2 and 3 as regards the relative number of large cells

Layers (8 unit zones)	Difference of percentages	Number of degrees of freedom	t value	Stat. sign
4 & 3	6 9—2 4	2206	2 90	h s
2 & 3	5 6—2 4	3214	4 64	v h.s

B Distribution in depth of integrated slow activity

When the animal was in a state of deep anesthesia (DA group see above) the slow activity varied considerably in frequency and amplitude Sometimes the slow waves occurred in fusiform series resembling Nembutal spindles recorded from the cortical surface Slow activity of irregular type was, however, more common The average duration of the single waves was 50—150 msec and the amplitude 0 5 — 1 5 mV Sometimes oscillations with higher frequency were superimposed upon the crests of the waves Slight mechanical movements of the brain may cause a distortion in the records and changes in the slow activity due to the shift of the electrode tip was indicated by the integrator Records in which it could be shown that oscillations were due to mechanical vibrations were omitted

The spontaneous activity obtained could be divided into the following components

1) Inherent noise caused by thermal noise at the electrode tip having an amplitude of 100—200 μV The variations of this noise were small because of the small variations in electrode impedance

2) Background noise consisting of irregular high frequency low amplitude activity which increased in a typical way when the electrode approached the active cell In several cases this buzzing could be shown to decrease when the electrode — according to the micrometer value — entered the white matter However, since this change in activity was not always distinct it was not used systematically for the estimation of the total depth of the claustro insular cortex

3) Spike potentials

4) Large slow waves

The inherent noise and the background noise were relatively constant and are included in the integration value which constitutes the basic level It was also found that the appearance of spike potentials had very little effect on the integration value

Thus the large amplitude slow waves play the dominant role in determination of the integration value, and the varying integration

values obtained at different stages of anesthesia were regarded to be due to the variations in this component.

In the majority of cases variations in the integration values were found to be due to variations in both amplitude and duration of the slow waves. As the integrator recorded activity of both polarities, the phase reversal often reported in studies of evoked responses did not appear in the results. When recordings were made during several 15 sec. periods successively with the same electrode position, the size of the integration value generally decreased with time.

In light anesthesia the slow activity was scanty and the sporadic slow waves of large size did not contribute appreciably to the integration values. In aging preparations variable and irregular slow waves with a duration of 300—500 msec. were recorded. In these cases there was no spatial differentiation of the slow activity.

Some typical examples will be presented in order to illustrate the distribution of the integrated activity in different cortical layers recorded at successive electrode insertions. The influence of the depth of anesthesia on the integrated activity in different cortical layers will also be described.

Fig. 5 shows the relative values for the integrated activity at different cortical depths obtained from three electrode insertions (I_1, I_2 and I_3). The electrode was moved in 10 μ steps down to a depth of 500 μ. The electrode impedance was constant.

Curve I_1 illustrates the situation in deep anesthesia. Curve I_2 was obtained in a later phase, when an additional dose of Nembutal had been injected and curve I_3 shows the spatial distribution of the slow activity some hours later. Judged by the narcotic level of the preparation, the values obtained in the first two series (I_1 and I_2) belong to the DA group (see above) and the values of the third series (I_3) to the LA group. The curve based on the integration values from the first insertion (I_1) show certain irregularities. This type of curve was often obtained in the initial phase of Nembutal anesthesia during which the slow activity is labile, as was mentioned above. The second insertion was made after an additional dose of Nembutal which was followed by an increase of the slow activity. The curve (I_2 in Fig. 5) showing the spatial distribution of the integrated activity obtained at the

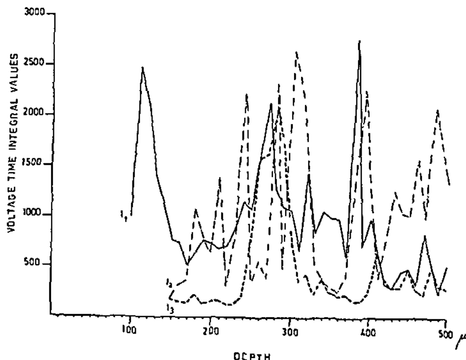


Fig 5 Relative voltage time integral values at different depths obtained from three successive electrode insertions (I_1 and I_2 belonging to the DA group and I_3 to the LA group) The electrode was moved in 10 μ steps down to a depth of 500 μ

second insertion shows a *maximal* peak at a depth of 300 μ This maximum is of special interest since it was found to be most typical for experiments of this type As seen in Table 1 the deep boundary of the first cortical layer is to be found at a depth of 320 ± 8.2 μ The highest peak having its maximum at 300 μ thus seems to correspond to the zonal lamina or the boundary between this layer and the first pyramidal cell layers proper (II and III in Table 1 and Fig 2) There is a corresponding dominating peak in the curve based on the values from the third insertion (I_3) the values of which indicate a diminished slow activity

The curves in Fig 6 based on values from two successive electrode insertions in another rabbit show the same general characteristics The values represented *by curve* I_1 *were obtained in a*

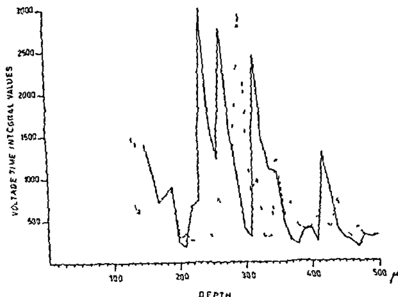


Fig 6 Relative voltage-time integral values at different depths obtained from two successive electrode insertions (I_1 belonging to the DA group and I_2 to LA group) The electrode was moved in 10 μ steps down to a depth of 500 μ

deep stage of anesthesia (DA) and the maximum of the curve corresponds to a depth of about 230 μ Curve I_2 in Fig 6 is based on values from the second insertion which was made in a stage of light anesthesia (LA) As seen the maximum of the curve corresponds to a depth of 300 μ

In Fig 7 the curves are based on values from three successive insertions in the claustrum the two first insertions (I_1 and I_2) being made in deep anesthesia (DA) and the third (I_3) in light anesthesia (LA) In this experiment the electrode was moved in 100 μ steps The curves based on the values from the first two insertions made in deep anesthesia are similar and their peak values are found at a depth of 2800—2900 μ, which depth corresponds to the deepest part of the claustrum As was the case in the experiments described above the slow activity was reduced

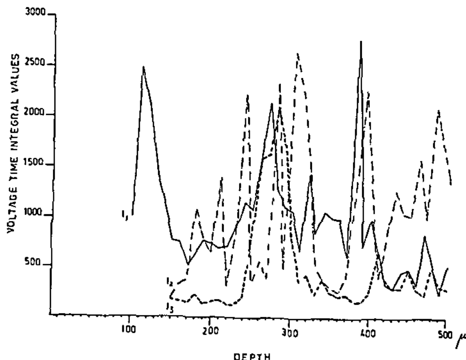


Fig 5 Relative voltage time integral values at different depths obtained from three successive electrode insertions (I_1 and I_2 belonging to the DA group and I_3 to the LA group) The electrode was moved in 10 μ steps down to a depth of 500 μ

second insertion shows a *maximal* peak at a depth of 300 μ This maximum is of special interest since it was found to be most typical for experiments of this type As seen in Table 1 the deep boundary of the first cortical layer is to be found at a depth of 320 ± 8.2 μ The highest peak having its maximum at 300 μ thus seems to correspond to the zonal lamina or the boundary *between this layer and the first pyramidal cell layers proper* (II and III in Table 1 and Fig 2) There is a corresponding dominating peak in the curve based on the values from the third insertion (I_3) the values of which indicate a diminished slow activity

The curves in Fig 6 based on values from two successive electrode insertions in another rabbit show the same general characteristics The values represented by curve I_1 were obtained in a

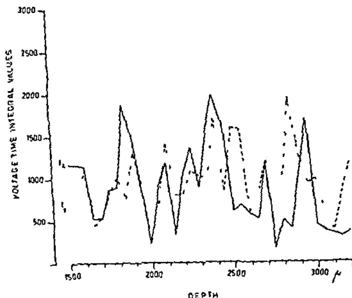


Fig 8 Relative voltage time integral values at different depths between 1500 μ and 3200 μ obtained in an experiment in which two successive insertions were made in light anesthesia (LA group) Curve I_1 shows the values obtained from the first insertion which was made in 50 μ steps and curve I_2 shows the values obtained at every 50 μ step from the second insertion which was made in 10 μ steps

in light anesthesia as indicated by curve I_2 the peak of which showed the same position as curve I_1 and I_2 around the depth of 2800—2900 μ

Fig 8 illustrates the integrated activity at varying depths between 1500 and 3200 μ obtained in an experiment in which two successive insertions were made in light anaesthesia Curve I_1 shows the values obtained in the first insertion which was made in 50 μ steps and curve I_2 the values obtained at every 50 μ step from the second insertion which was made in 10 μ steps Insertion 1 (curve I_1) took 30 minutes and insertion 2 (curve I_2) 90 minutes The experiment shows that the curves obtained on the basis of values from two insertions made at the same anesthetic level but with different time course have similar general shape

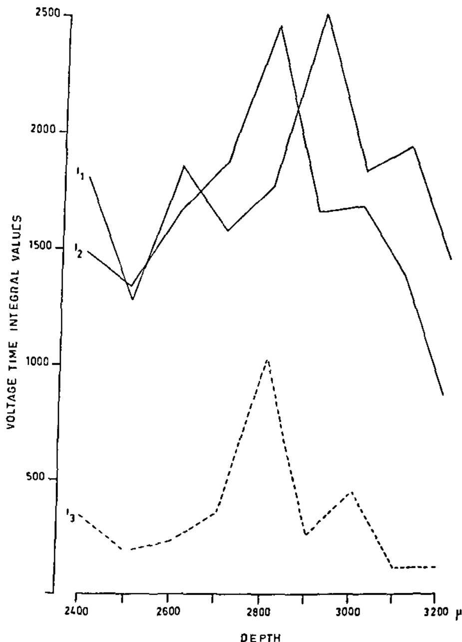


Fig 7 Relative voltage time integral values at different depths between 2400 and 3200 μ obtained from three successive electrode insertion I_1 and I_2 belonging to the DA group and I_3 to the LA group) The electrode was moved in 100 μ steps

Table 7 Integration value per unit zone (see text) at different depths (mean, standard deviation and standard error) 17 electrode insertions LA group

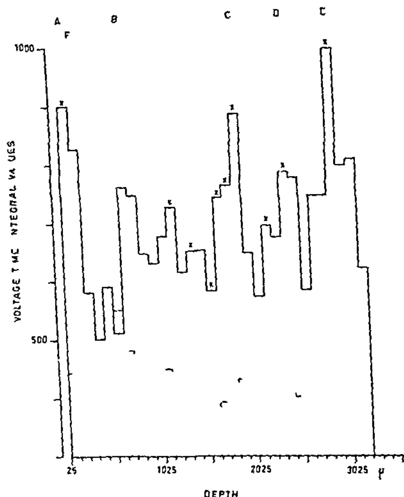
Zone (μ)	Mean $\bar{x}$	St D s_x	St E $s(\bar{x})$
125	460	330	82
225	700	650	158
325	570	790	191
425	480	430	104
525	400	250	59
625	500	520	120
725	450	370	91
825	440	290	70
925	490	390	92
1025	430	260	63
1125	450	430	105
1225	400	270	66
1325	470	340	82
1425	350	150	45
1525	380	370	90
1625	360	220	54
1725	390	290	70
1825	370	270	66
1925	430	330	81
2025	440	380	93
2125	360	300	72
2225	350	210	51
2325	340	263	64
2425	410	330	80
2525	400	480	116
2625	400	390	98
2725	440	400	98
2825	510	430	103
2925	420	390	95
3025	420	410	100
3125	350	180	53
3225	350	350	116

Tables 6, 7 and 8 show the mean integration values and the differences of the means for both groups. Fig. 9 shows the distribution in depth of the mean integration values for both groups (DA group represented by full lines and LA group by dashed lines).

Table C. Integration value per unit zone (see text) at different depths (mean, standard deviation and standard error). 14 electrode insertions. DA group

Zone (μ)	Mean $\bar{x}$	St. D s_x	St. E $s\{\bar{x}\}$
125	900	590	164
225	830	630	167
325	580	390	105
425	500	240	64
525	590	450	120
625	510	350	93
725	760	530	141
825	750	640	171
925	650	390	105
1025	630	280	74
1125	680	460	122
1225	730	440	119
1325	630	440	118
1425	650	470	126
1525	650	490	131
1625	580	270	73
1725	740	400	107
1825	760	330	102
1925	880	680	183
2025	650	370	98
2125	570	320	86
2225	690	380	102
2325	670	690	184
2425	780	460	122
2525	770	760	203
2625	580	520	139
2725	740	610	170
2825	740	470	135
2925	990	850	257
3025	790	820	248
3125	800	760	269
3225	600	420	148

The material treated statistically comprised data from 14 electrode insertions (9 animals) from the DA group and 17 (9 animals) from the LA group. Recording data from a maximum of three electrode insertions per animal was included in the material



F g 9 D tribution n depth of the mean integrat on values for group D\ (full l ne) and for group LA (dashed lne) The symbol x) s g- f e r nc n wh ch tl e difference between the mean DA and LA ntegrat on values is statist cally s gnif cant \ B C D E and F represent the ma n peaks

Table 8 Evaluation of the statistical significance of the difference between the DA and LA groups as regards the integration values per unit zones at different depths

Zones (μ) (DA & LA)	Difference of means	Number of degrees of freedom f	t value	Stat sign
125	900—460	27	2 58	s
225	830—700	29	0 56	—
325	580—570	29	0 07	—
425	500—480	29	0 21	—
525	590—400	29	1 50	—
625	560—510	29	0 30	—
725	700—480	29	1 74	—
825	750—440	29	1 76	—
925	650—490	29	1 14	—
1025	630—430	29	2 02	—
1125	680—450	29	1 40	—
1225	730—400	29	2 49	s
1325	630—470	29	1 14	—
1425	650—380	29	2 22	s
1525	650—380	29	1 74	—
1625	580—360	29	2 46	s
1725	740—390	29	2 82	h s
1825	760—370	29	3 34	h s
1925	880—430	29	2 42	s
2025	650—440	29	1 50	—
2125	570—360	29	1 89	—
2225	690—350	29	3 18	h s
2325	670—340	29	1 83	—
2425	780—410	29	2 60	s
2525	770—400	29	1 64	—
2625	580—400	28	1 07	—
2725	740—440	28	1 62	—
2825	740—510	27	1 38	—
2925	990—420	28	2 38	s
3025	790—420	26	1 59	—
3125	800—350	15	1 76	—
3225	600—380	15	1 17	—

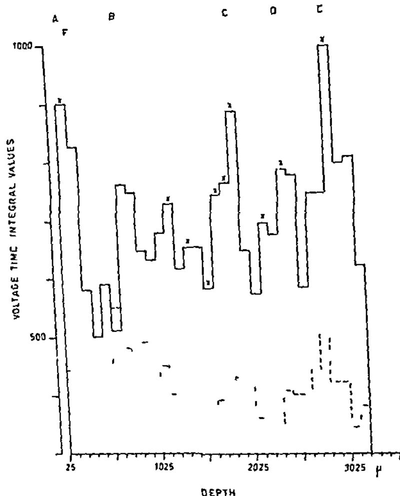


Fig. 9 Distribution in depth of the mean integration values for group DA (full line) and for group LA (dashed line) The symbol x) signifies zones in which the difference between the mean DA and LA integration values is statistically significant. A, B, C, D, E and F represent the main peaks

Table 9 Evaluation of the statistical significance of the principal peaks in diagram 9 showing integration value per unit zone at different depths DA and LA groups

Anesth group	Peak in diagram 9	Zones (μ)	Difference of means	Number of degrees of freedom f	t value	Stat sign
DA	A	125 & 425	900—500	25	2.35	s
		125 & 325	900—580	26	1.75	—
	B	725 & 625	760—510	26	1.49	—
		725 & 1025	760—630	26	0.84	—
	C	1925 & 1625	880—580	26	1.52	—
		1925 & 2125	880—570	26	1.53	—
	D	2425 & 2125	780—570	26	1.36	—
		2425 & 2625	780—580	26	1.07	—
	E	2925 & 2625	990—580	23	1.47	—
		2925 & 3225	990—600	17	1.17	—
LA	F	225 & 125	700—460	31	1.32	—
		225 & 525	700—400	32	1.78	—

The difference in general course between the two distribution curves is notable except for the area near the surface. The symbol x in Fig 9 signifies zones in which the differences between the mean integration values are statistically significant. Thus, Nembutal, like many other anesthetics, enhances slow spontaneous activity. It was therefore expected that the integration values obtained from these two groups should differ. It should be pointed out that the DA group does not represent recordings made under very heavy anesthesia in which the slow wave activity decreases.

Table 9 shows the statistical evaluation of some the most significant peaks in the integration curves. The only significant peak value was that found near the surface in the DA group corresponding to the *zonal lamina*. The variation of the integration values was to great to permit a further accurate analysis along this line.

C The distribution in depth of positive spike potential activity

Although main attention was paid to positive spike potentials it should be pointed out that negative spike potentials were also seen The number of these initially negative spike potentials clearly distinguishable from the base line was too small to permit a systematic study Generally the negative spike potentials were mostly obtained at depths of 1000—2000 μ

In the majority of cases the positive spike potentials — or more accurately the positive negative and monophasic positive potentials — were clearly distinguished from the base line They were generally characterized by a large amplitude and consequently were suitable for a systematic study The material was collected concurrently with the integration values

The total number of positive spike potentials in the material was 164 59 being obtained in the animals under deep anesthesia (DA) and 105 in the animals under light anesthesia (LA)

The relative number of recordings (duration 15 sec) including a positive spike potential (in per cent of all recordings) is given in Table 10 Fig 10 and 11 show the probability for the appearance of positive spike potentials in the DA and LA groups at different depths The distribution curves resemble each other in their general configuration but display certain differences The maximum appears in both groups at a depth of about 1000 μ, corresponding to layer V_b In both groups the peak is preceded by an abrupt rise and followed by a gentle general fall in the curve In general the distribution curves of the DA and LA groups are almost identical from the surface to a depth of about 1030 μ but from there on the curves show that in the deeper layers a greater number of positive spike potentials were obtained in light than in deep anesthesia

The distribution curves of both groups show local accumulations of spike activity the statistical significance of which is shown in Table 11 According to this table the only statistically significant accumulation in the DA group was localized at a depth of around 1000 μ i e in the layer of the large pyramidal

Table 10 Number of 15 sec recordings including a positive spike potential per unit zone at different depths and number of positive spike recordings in per cent of all recordings at different depths DA group (14 electrode insertions) and LA group (17 electrode insertions)

Zone (μ)	DA group			LA group		
	Number of positive spike recordings	Per cent	St E $s\{p\}$	Number of positive spike recordings	Per cent	St. E $s\{p\}$
125	—	—	—	—	—	—
225	—	—	—	—	—	—
325	2	7 2	4 9	2	5 9	4 0
425	1	3 6	3 5	4	11 8	5 5
525	4	14 3	6 6	3	8 8	4 9
625	3	10 7	5 9	3	8 8	4 9
725	3	10 7	5 9	3	8 8	4 9
825	1	3 6	3 5	5	14 7	6 1
925	4	14 3	6 6	2	5 9	4 0
1025	6	21 4	7 8	5	14 7	6 1
1125	5	17 9	7 3	3	8 8	4 9
1225	3	10 7	5 9	8	23 6	7 3
1325	1	3 6	3 5	2	5 9	4 0
1425	3	10 7	5 9	2	5 9	4 0
1525	2	7 2	4 9	7	20 6	6 9
1625	1	3 6	3 5	7	20 6	6 9
1725	3	10 7	5 9	2	5 9	4 0
1825	2	7 2	4 9	2	5 9	4 0
1925	2	7 2	4 9	4	11 8	5 5
2025	—	—	—	2	5 9	4 0
2125	3	10 7	5 9	5	14 7	6 0
2225	1	3 6	3 5	7	20 6	6 9
2325	1	3 6	3 5	3	8 8	4 9
2425	1	3 6	3 5	2	5 9	4 0
2525	1	3 6	3 5	3	8 8	4 9
2625	1	3 6	3 5	5	15 7	6 4
2725	2	7 7	5 2	4	11 8	5 5
2825	—	—	—	3	8 8	4 9
2925	1	4 6	4 4	3	8 8	4 9
3025	—	—	—	1	2 9	2 9
3125	1	6 3	6 1	3	16 7	8 8
3225	1	6 3	6 1	—	—	—

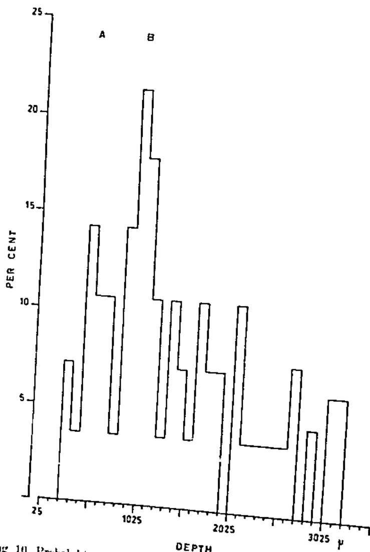


Fig 10 Probability of appearance of positive spike potentials in the *D* 1 *group* at different depths The probability expressed as per cent of 15 sec recordings that showed a positive spike potential

Table 10 Number of 15 sec recordings including a positive spike potential per unit zone at different depths and number of positive spike recordings in per cent of all recordings at different depths DA group (11 electrode insertions) and LA group (17 electrode insertions)

Zone (μ)	DA group			LA group		
	Number of positive spike recordings	Per cent	St E s{p}	Number of positive spike recordings	Per cent	St E s{p}
125	—	—	—	—	—	—
225	—	—	—	—	—	—
325	2	7 2	4 9	2	5 9	4 0
425	1	3 6	3 5	4	11 8	5 5
525	4	14 3	6 6	3	8 8	4 9
625	3	10 7	5 9	3	8 8	4 9
725	3	10 7	5 9	3	8 8	4 9
825	1	3 6	3 5	5	14 7	6 1
925	4	14 3	6 6	2	5 9	4 0
1025	6	21 4	7 8	5	14 7	6 1
1125	5	17 9	7 3	3	8 8	4 9
1225	3	10 7	5 9	8	23 6	7 3
1325	1	3 6	3 5	2	5 9	4 0
1425	3	10 7	5 9	2	5 9	4 0
1525	2	7 2	4 9	7	20 6	6 9
1625	1	3 6	3 5	7	20 6	6 9
1725	3	10 7	5 9	2	5 9	4 0
1825	2	7 2	4 9	2	5 9	4 0
1925	2	7 2	4 9	4	11 8	5 5
2025	—	—	—	2	5 9	4 0
2125	3	10 7	5 9	5	14 7	6 0
2225	1	3 6	3 5	7	20 6	6 9
2325	1	3 6	3 5	3	8 8	4 9
2425	1	3 6	3 5	2	5 9	4 0
2525	1	3 6	3 5	3	8 8	4 9
2625	1	3 6	3 5	5	15 7	6 4
2725	2	7 7	5 2	4	11 8	5 5
2825	—	—	—	3	8 8	4 9
2925	1	4 6	4 4	3	8 8	4 9
3025	—	—	—	1	2 9	2 9
3125	1	6 3	6 1	3	16 7	8 8
3225	1	6 3	6 1	—	—	—

Table 11 Evaluation of the statistical significance of the principal peaks in diagrams 10 and 11 showing number of positive spike recordings in per cent of all recordings per unit zone at different depths DA and LA groups

Anesth group	Peak in diagram 10 (DA) respect 11 (LA)	Zones (μ)	Difference of percentages	Number of degrees of freedom f	t value	Stat sign
DA	A	525 & 425	14 3—3 6	54	1 43	—
		525 & 625	14 3—10 7	54	0 41	—
	B	1025 & 825	21 4—3 6	54	2 10	s
		1025 & 1325	21 4—3 6	54	2 10	s
LA	A	1225 & 1125	23 6—8 8	66	1 69	—
		1225 & 1325	10 7—3 6	66	2 12	s
	B	1525 & 1425	20 6—5 9	66	1 83	—
		1525 & 1725	20 6—5 9	66	1 53	—
	C	2225 & 2025	20 6—5 9	66	1 83	—
		2225 & 2425	20 6—5 9	66	1 83	—
	D	2625 & 2425	15 7—5 9	64	1 30	—
	E	3125 & 3025	16 7—2 9	50	1 49	—

cells Prominent though not statistically significant peaks occurred in the LA group also at the 1500 and 2200 μ depths

With the method used no positive spike potentials were obtained from the surface layers down to a depth of 200 μ in either group It should however be mentioned that negative spike potentials could be found in this layer

Large positive spike potentials with an amplitude of more than one millivolt which could easily be recorded with the aid of an electronic counter were dealt with separately These potentials were also either positive negative or monophasic positive The duration of the negative deflections varied and when the duration of the negative phase was at it greatest the configuration of the potential was as shown in Fig 12 The average frequency of such potentials in the DA group was 1—2 per sec and in the LA group 4—5 per sec Since recordings which displayed positive spike

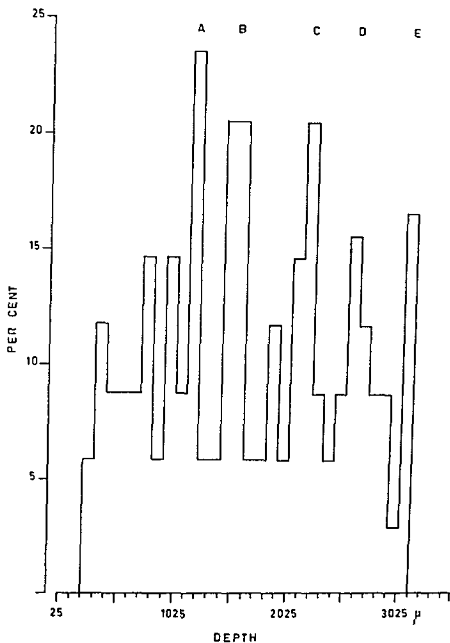


Fig 11 Probability of appearance of positive spike potentials in the *L 1 group* at different depths

Table 12 The proportion of large positive spike recordings in per cent of all recordings per 8 unit zones DA and LA groups

Anesth. group	8 unit zones		Number of large positive spike recordings	Per cent	St E $s\{p\}$
	No	Zones (μ)			
DA	1	from 125 to 825	6	2.71	1 08
	2	from 925 to 1625	9	4 02	1.31
	3	from 1725 to 2425	4	1 79	1 33
	4	from 2525 to 3225	1	0 55	0 55
LA	1	from 125 to 825	1	0 37	0 37
	2	from 925 to 1625	11	4 05	1.20
	3	from 1725 to 2425	8	2 94	1 03
	4	from 2525 to 3225	10	4 20	1.30

Table 13 Evaluation of the statistical significance of the differences between layers 1_{DA} and 1_{LA} and between layers 4_{DA} and 4_{LA} as regards the percentages of large positive spike recordings

Layers (8 unit zones)	Difference of percentages	Number of degrees of freedom f	t value	Stat sign
1_{DA} & 1_{LA}	2 71—0 37	490	2 04	s
4_{DA} & 4_{LA}	4 20—0 55	418	2 50	h s

shown by Fig 13 is of special interest According to Table 13 this difference is highly significant Fig 13 thus shows that the large claustral cells exhibit a pronounced spontaneous activity of this type in light anesthesia as compared with the state of deep anesthesia No such difference is found in zone 2 corresponding to layer V On the contrary the spontaneous activity in this layer is of the same intensity in light and deep anesthesia Thus, the large insular pyramidal cells in layer V e do not react to changes in anaesthesia as critically as the large claustral cells

The spatial extent of the proximity potentials is regarded not to be much larger than the diameter of the soma which serves as

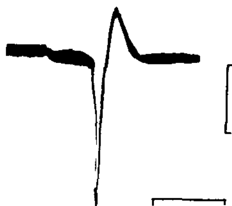


Fig 12 Configuration of an initial positive diphasic spike potential Calibration Time bar 10 msec Voltage bar 0 5 mV

potentials with an amplitude over one millivolt were relatively few (20 in the DA group and 30 in the LA group) the 8 unit zones were used with the total cortical depth being divided into four zones 25—825 μ 825—1625 μ 1625—2425 μ and 2425—3225 μ Fig 13 shows the probability for the appearance of such potentials in these different zones obtained from all recordings (15 sec duration) The diagram indicates that the probability for *the appearance of the large positive potentials at different depths* is different in the two groups In deep anesthesia these potentials appear most frequently in zone 2 whereas in light anesthesia they appear most frequently in zone 4

The number of recordings with a large positive spike potential in per cent of all recordings made from the different zones in both groups are given in Table 12 Table 13 shows the statistical significance of the difference between the groups as regards the probability of the appearance of the large positive spike potentials in zones 1 and 4 Fig 13 shows that these large positive spike potentials tended to appear more often in the zones 2 and 4 than in the intervening ones The similarity between the diagram in Fig 13 with that in Fig 4 showing the distribution of large cells at different depths indicates that the main origins of the large *spike potentials are situated in layers containing the largest peri* karyon The difference between the LA and DA group in zone 4

origin (Li and Jasper 1953). Therefore they may not be found when the electrode is moved in 50 μ steps They are more likely to be observed when the distances between the recording points are only 10 μ Using such a procedure would, however, take too long time for a comparison of the results obtained near the surface with those obtained from the depth (see above) Such an examination was therefore limited to some few layers only It was possible to record the same spike potential in the majority of cases at three successive recording points, i e over a distance of 30 μ The greatest recording distance within which the same potential could be recorded was found to be 70 μ, within the claustral area

D Correlation between the density of claustro insular cells and the electrophysiological data

Table 14 in which the morphological and functional data are presented shows that there is a highly significant positive correlation between the probability for the appearance of positive spike potentials and the density of cells in the DA group The table also shows that there is a statistically significant negative correlation between the integrated slow activity and the density of cells in the DA group In the LA group on the other hand the correlation between the electrophysiological data and the density of cells is not statistically significant

Table 14 Correlations between the density of claustro-insular cells and the electrophysiological data (PSP a = positive spike potential activity INT a = integrated activity) In all cases the number of degrees of freedom = 28

Factors participating in the correlation	Correlation coefficient r	t value	Stat sign
Cells/PSP a DA	+ 0.51	3.28	h s
Cells/INT a DA	− 0.37	2.18	s
Cells/PSP a LA	+ 0.26	1.43	—
Cells/INT a LA	− 0.20	1.14	—

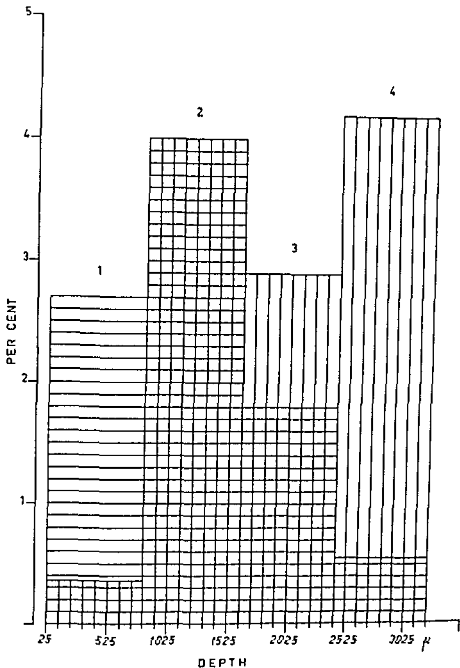


Fig 13 Probability of appearance of large positive spike potentials in different layers (8 unit zones) DA and LA group

depths solely slow activity may occur whereas positive spike potentials and less slow activity is characteristic for intervening layers However, in some experiments pronounced slow activity and positive spike potentials appeared during the same 15 sec period In these cases, however, the electrode position was found to be critical A small change in the position was enough to cause a complete change in pattern

The above correlations are illustrated graphically in Fig 14 which gives the values from one electrode insertion made with 10 μ steps The insertion is the same as in Fig 8 (insertion 1) Every integration value obtained is plotted and the positive spike potentials observed are marked The diagram illustrates data obtained from depths between 2000—3000 μ, corresponding to the claustrum Fig 14 shows that positive spike potentials occur at depths between 2490—2640 μ and between 2920—3080 μ and that the integration values are reduced at the corresponding depths At a depth of 2000—2300 μ however, the integration values are low although no positive spike potentials are recorded simultaneously

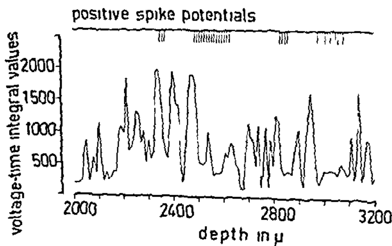


Fig 14 Integration values at different depths between 2000 and 3200 μ obtained from one electrode insertion The electrode was moved in 10 μ step The insertion is the same as in Fig 8 (I_1) The depths at which positive spike potentials were obtained are indicated by arrows

E. Correlation between integrated activity and positive spike potential activity

From what has been said above a negative correlation between the positive spike potentials and the integrated slow activity is to be expected in the DA group. Table 15 shows that there is actually a statistical significance of this negative correlation both in the LA and DA group. If, on the other hand, the correlations in the two groups are examined crosswise, as shown in Table 15, the statistical value of the correlation coefficient is not significant.

The data obtained above can examined also by means of partial correlation (see Methods). If positive spike potential activity and integrated activity are correlated under the assumption that the cell density remains constant throughout whole cortical depth, the value of the coefficient (PSP a/INT a) changes in group DA to −0.23 (difference 0.14) and loses its statistical significance. In the LA group this change is smaller and the value of the coefficient becomes −0.37 (difference 0.03), which is still significant.

The weight of the negative correlation must be assessed from the standpoint of the method used. Integrated activity and spike potential activity were studied simultaneously during periods with a duration of 15 seconds. As has been pointed out the potential pattern remains the same at a certain point during the time for recording and it has also been found that at certain

Table 15. Correlations between integrated activity (INT a) and positive spike potential activity (PSP a). In all cases number of degrees of freedom = 28

Factors participating in the correlation	Correlation coefficient r	t value	Stat. sig.
$PSP\ a_{DA}/INT\ a_{DA}$	−0.37	2.18	s
$PSP\ a_{LA}/INT\ a_{LA}$	−0.40	2.41	s
$PSP\ a_{DA}/INT\ a_{LA}$	−0.09	0.49	—
$PSP\ a_{LA}/INT\ a_{DA}$	−0.22	1.24	—

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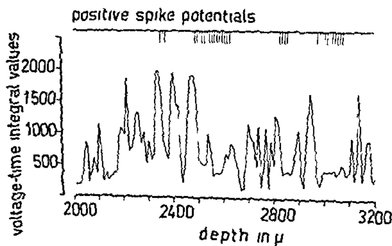


Fig 14 Integration values at different depths between 2000 and 3200 μ obtained from one electrode insertion The electrode was moved in 10 μ steps The insertion is the same as in Fig 8 (I_2) The depths at which positive spike potentials were obtained are indicated by arrows

E. Correlation between integrated activity and positive spike potential activity

From what has been said above a negative correlation between the positive spike potentials and the integrated slow activity is to be expected in the DA group. Table 15 shows that there is actually a statistical significance of this negative correlation both in the LA and DA group. If, on the other hand, the correlations in the two *groups* are examined crosswise, as shown in Table 15, the statistical value of the correlation *coefficient* is not significant.

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spontaneous and not as the result of mechanical stimulation of the cell by the electrode tip It is obvious that for a frequency study this factor would be of importance However the aim of these investigations has not been to study the frequency characteristics of the cortical cells and the conclusions are not based on any data concerning discharge frequency

The typical injury potentials which appear in trains with a high accelerating frequency have always been exluded from this material However it has been suggested that the appearance of initial positive spikes alone would indicate that the cell from which that spike activity is led off is damaged Thus MOUNTCASTLE *et al* stated that the absence of slow background activity tends to be associated with initially positive spike potentials The appearance of such a discharge is always accompanied by an increased degree of isolation i e by a marked decrease in the background neuronal activity recorded (MOUNTCASTLE *et al* 1957) This could mean that when initially positive spike potential activity is recorded the conditions are unfavourable to obtain slow activity due to damage of the cells in the vicinity of the electrode tip One could thus argue that the negative correlation between integrated slow activity and positive spike potential activity may be explained on this basis

On the other hand it has been shown that the positive polarity of a recorded spike potential does not necessarily indicate cell injury if the frequency of the spike potentials does not rise to the so called injury level Several workers have reported that the same positive spike potential can be recorded at a certain depth even if the electrode is moved in the vicinity of the cell (GRANIT & PHILLIPS 1956 MOUNTCASTLE *et al* 1957) MOUNTCASTLE and co-workers (1957) have also suggested the possibility that such an initially positive spike potential represents capacitive recording with the tip of the electrode pressing against the cell membrane The appearance of isolated initially positive spike potentials without any background activity may also indicate that the electrode tip has attained a critical position between a region characterized by giving a graded slow potential and a region characterized by giving an all-or none type spike potential (see CLARE & BISHOP 1955 b)

VI. DISCUSSION

The present study of the different types of electrical activity in the various cortical layers is based on histological studies of nerve cell density and electrophysiological data obtained from experiments in which the depth of the microelectrode tip was estimated from a micrometer scale on the micromanipulator. When comparing such histological and functional data, one must consider errors which may be introduced by structural changes in the histological preparations and by difficulties in the estimation of the exact position of the electrode tip (e.g. dimpling of the cortical surface, non perpendicular direction of the electrode insertion etc.). However, it is known that the positive spike potentials are readily obtained from layers rich in nerve cells and in the present material a highly significant correlation was found between the probability of the appearance of positive spike potentials and cell density at different depths. Therefore it can be assumed that the measurements of the depths of the electrode tip are equivalent to the depth measurements obtained from the histological preparations.

The statistical treatment of the results obtained from the animals under deep anesthesia shows a negative correlation between the integrated slow activity and the positive spike potential activity, the latter being obtained from the perikaryon rich layers. Before discussing this negative correlation the significance of the positive spike potentials should be considered.

In these investigations the electrode was always moved in fixed steps rather than continuously in order to enable the studies of the correlation of the functional and structural data to be based on a material which could be treated statistically. Under these experimental conditions it is not known to what extent the recorded positive spike potential may be considered

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This difference in the distribution of spike activity and slow activity which seems to exist between light and deep anesthesia needs not concern the superficial and deep cortical layers uniformly. The large insular pyramidal cells in layer V c do not react to changes in anesthesia as critically as the large claustral cells, as was mentioned on page 45. The last mentioned observation points to a differential behaviour of these two neuron structures which are both characterized by a high density of large nerve cells.

According to DEMPSEY and MORISON (1942 a b) the typical spindle activity in animals under barbiturate anesthesia follows the same functional laws as the recruiting phenomenon. This phenomenon is dependent on activation mediated by the so-called unspecific thalamo-cortical pathways. According to LORENTE DE NO (1951) the distribution of the unspecific thalamo-cortical afferents is different from that of specific afferents. He claimed that the net work of unspecific fibres terminates in the cortex diffusely in contrast to the relatively precise localization of the specific thalamo-cortical pathways. Electrophysiological results pointing in the same direction have been obtained in microelectrode studies by LI *et al.* (1956). They suggest the possibility that dendritic depolarization may occur in response to unspecific afferent volleys even without discharge of the cortical cells.

It may thus be that the axo-dendritic connections of the unspecific pathways in the claustro-insular area influence the distribution in depth of integrated slow activity.

The negative correlation between *the integrated slow activity* and the positive spike potentials would thus mean that in deep anesthesia the slow activity is less pronounced in the perikaryon rich layers than in the intervening layers characterized by a low cell density. Thus there ought to be a negative correlation between *the integrated slow activity and the cell density in deep anesthesia*. Although the results of the individual experiments presented in chapter V b suggest that this is the case, statistical significance could not be obtained except for the superficial layer. The lack of statistical significance may be due to several factors. The recordings were *not made simultaneously from all the different points* at varying depths and the slow activity at different levels may change somewhat in the course of the experiment. The relatively short recording time may be another reason for the statistical *variations. A recording time of 15 seconds was chosen as the result* of a compromise. *A prolongation of the recording time would have* lengthened critically the time for the total experiment and thus impaired the comparability of the results obtained from the surface and from the deeper structures. DROCHOCKI (1956) studied the integrated E.E.G. activity in a cortical area and observed that the variations disappeared almost completely in successive recordings when the recording time was lengthened to 200—300 sec. Finally the integrated slow activity may be heterogeneous and the relation between its different components may change with cortical depth.

An increase in activity of short duration was frequently observed after the electrode had been moved to a new position. This increase of activity was assumed to be due to mechanical stimulation caused by the electrode tip and possibly also due to the injury of some units. Therefore integration *was not started until* 5 seconds after moving the electrode, during which interval the activity reached a constant level.

Variations in the depth of anesthesia within the range tested had no significant effect on the negative correlation between positive spike *potential* activity and *integrated* slow activity. However, the positive correlation between spike potential activity and cell density is significant in deep anesthesia only. It follows that the negative correlation between integrated slow activity and cell density is significant in deep anesthesia only.

sections from 28 animals which were not used in the electrophysiological experiment (see Histological preparation in Chapter IV)

6 Diagram in which the number of cells per unit zone (see page 25) was plotted against depths in μ illustrate the distribution of the counted nerve cells in the different cortical layers The material was treated statistically (Chapter V A)

7 On the basis of the electrophysiological data obtained the correlation between the appearance of the slow activity and spike potentials was studied (Chapter V E) A significant negative correlation was found between the appearance of slow activity and spike potentials both in light (r = —0 40) and in deep anaesthesia (r = —0 37)

8 On the basis of the electrophysiological and the histological material the distribution of the spike and slow wave activities was correlated with the density of claustro insular cells

9 A positive correlation between spike activity and cell density was found to be highly significant in the animals under deep anesthesia (r = +0 51) This positive correlation was non signif icant in the animals under light anesthesia (r = +0 26)

10 A significant negative correlation was found between the slow activity and the cell density in the animals under deep anesthesia (r = —0 37) This negative correlation was non-significant in the animals under light anesthesia (r = —0 20)

11 The difference between light and deep anesthesia as regards the distribution of spike activity and slow activity seems not to concern the superficial and deep cortical layers uniformly since it was found that the large insular pyramidal cells in layer V C did not react to changes in anesthesia as critically as the large claustral cells

12 On the basis of the results obtained the dendritic origin of the slow wave activity correlated to the layers characterized by a low cell density was discussed

VII. SUMMARY

1. In the present investigation the distribution of the spike and slow wave activities was studied with an extracellular micro electrode technique in different layers throughout the entire cortical depth. The claustro insular area in the rabbit was chosen as the object for this study and a description of this area (*area insularis agranularis anterior dorsalis*) is presented in Chapter III.
2. The spike and slow wave activity was led off with a KCl filled glass capillary electrode which was inserted in stepwise fashion to different depths in the claustro insular area. Each *insertion was made in 50 μ steps perpendicular to the cortical* surface, the depth of the electrode tip under the surface being estimated by the aid of a micrometer attached to the micro manipulator. The slow potentials at each electrode position were integrated electronically during *fixed intervals* of time and the probability of the appearance of positive spike potentials during the same time periods was estimated (see Recording in Chapter IV.)
3. Diagrams in which the time integral values for the slow wave activity and the probability of appearance of positive spike potentials were plotted against depths in μ show the distribution in depth of these two types of cortical activity. The material was treated statistically (Chapters V B and V C).
4. The distribution in depth of the two types of activity was studied on animals under light anesthesia (LA group, see page 15) and on animals under deep anesthesia (DA group, see page 15).
5. *The density at different* cortical depths of the claustro insular cells, the projections of which exceeded 80 μ² (see page 18) was studied on histological preparations obtained from perpendicular

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KAROLINSKA INSTITUTET (FACULTY OF MEDICINE
STOCKHOLM SWEDEN

CATECHOLAMINE PRODUCTION AND RELEASE IN EXPOSURE AND ACCLIMATION TO COLD

BY

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MEDICAL RESEARCH FELLOW NATIONAL RESEARCH COUNCIL, CANADA

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TO MY WIFE AND OUR CHILDREN

CONTENTS

INTRODUCTION

In the "*Leçons sur la chaleur animale*" given in 1871—1872, Claude Bernard demonstrated that heat production on exposure to cold was referable to two main sources: muscular activity and chemical processes other than those concerned with muscular activity. It appears now that identification of these chemical factors and elucidation of their mechanisms of action would be the key to understanding the physiology of cold defense.

Since the pioneer works of Cannon and his associates several studies have shown an increased sympatho-adrenal activity in animals exposed to cold. It has first been postulated that the chemical regulation of heat production might be mediated through the release of adrenaline from the adrenal glands. Recently, emphasis has been put on the possible role of noradrenaline as the mediator in the chemical regulation of heat production. Whether or not other catecholamines known to occur normally in the organism, such as dopamine, are involved in these chemical processes is at the present not known.

It was then felt important to study more extensively the relation between catecholamines and exposure to cold. Special attention has been given to the production and release of these amines in response to acute and chronic cold stress by measuring their excretion in urine. Many questions pertinent to this problem have, of course, arisen during the course of these experiments. How was the pattern of the catecholamine excretion in the cold altered by previous exposure to a low environmental temperature? What were the sites of origin of these amines? Did cold exposure and acclimation affect the catecholamine concentrations in tissues? Was there some limitation in the catecholamine production and/or release? What were the respective roles of noradrenaline, adrenaline and dopamine in the response to cold stress and in the acclimation process? The present paper deals with experiments performed in order to answer some of these questions.

CHAPTER I

HISTORICAL SURVEY
CATECHOLAMINES AND EXPOSURE TO COLD

During exposure to cold the normal body temperature of the homeotherm is maintained, or the animal fails to survive. Consequently, heat loss must be decreased or heat production increased in order to maintain the balance. Several mechanisms may act to bring about a decreased heat loss. By far the most important, especially during short periods of exposure, is the peripheral vasoconstriction which reduces transference of heat from deep body tissues to the surface and in effect increases the insulating value of subcutaneous tissues. The increase in heat production, on the other hand, can be brought about by two means. The first or physical method consists of gross muscular activity and shivering. The second or chemical method is mediated by increased hormonal secretion, enzymatic adaptations and other biochemical modifications which increase heat production independently of muscular contraction. The effects of catecholamines in the response to cold stress can be ascribed to their vasoconstrictor action as well as to their stimulating effect on metabolism.

A large number of studies have shown an increased suprarenal adrenaline secretion in animals exposed to cold, as measured by its physiological effects on the denervated pupil or heart and on the glycemia. HARTMAN, MCCORDOCK and LODER (1923) reported that if cats were exposed to cold the width of the totally denervated pupil increased. Under the same conditions adrenalectomized animals showed no or only slight dilatation of the pupil. Similarly, the cardioacceleration of the denervated heart on cooling caused either by cold air or by cold water introduced into the stomach was almost completely abolished after exclusion of the adrenal medullary secretion (CANNON, QUERIDO, BRITTON and BRIGHT 1927). In a series of experiments on unanesthetized dogs exposed to cold air from + 9° to — 6° C, MORIN (1946, 1948) also showed an increased secretion of adrenaline from the adrenal medulla by measuring the rate of the denervated heart and the glycemia in normal and demedullated animals.

These results are at variance with those of the Japanese workers showing that catecholamines from extra-medullary sources are liberated in considerable amounts as a result of cold stimuli. The denervated heart of dogs accelerated on cooling by introduction of cold water into the stomach, and demedullation did not significantly affect the tachycardia (WADA 1935, NAGAKURA 1949). However, when animals were submersed in an ice-bath causing a large decrease in the body temperature, the demedullation of adrenals definitely reduced the cardioacceleration and the blood pressure elevation (WADA and FUZII 1940).

This physiological evidence for an increased secretion of adrenaline in animals exposed to cold has been confirmed by direct estimations of the suprarenal secretion in dogs (WADA, SEO and ABE 1935, SAITO 1928) These results, obtained in unanesthetized animals, emphasize the important point that the adrenaline secretion from the adrenals is related to the intensity of cold stimuli When cooling caused a considerable fall in body temperature there was a marked increase in the adrenaline secretion Thus, on exposing the normal dog to cold water at 0 5° — 1 0° C for 20 to 40 minutes, so that the rectal temperature fell to about 30° C, there was always a large increase in the adrenaline secretion (WADA, SEO and ABE 1935) On the other hand, when the body temperature fell by 0 5° to 1 7° C by subjecting the dog to superficial cold, little or no acceleration in the adrenaline secretion was detectable For instance, immersion of the dog in water of 5° to 7° C or placing the animal on a block of ice for 30 minutes did not increase the rate of adrenaline secretion, but introduction of cold water at 5° C into the stomach in amounts of 1,000 to 1,500 ml, causing a small decrease in body temperature, brought about a slight increase in the adrenaline secretion (SAITO 1928) These experiments on unanesthetized dogs clearly show that cold stimuli have to be of a relatively strong intensity in order to induce an adrenaline secretion from the adrenal glands

In cooled anesthetized dogs KLEPPING, TANCHE and CIER (1957) showed a definite increase in the secretion of medullary catecholamines, measured in the suprarenal venous blood, with an increase in the adrenaline percentage In contrast, HUME, EGDAHL and NELSON (1956) noted a marked and progressive decrease in the secretion of adrenaline and noradrenaline in adrenal venous blood of anesthetized hypothermic dogs The adrenaline outflow was reduced 10 fold at 26° C and 100 fold at 21° C However, there was a rapid increase in secretion of amines on re warming It is interesting to note that WADA, SEO and ABE (1935) also found that immediately after withdrawal of the animal from the cold bath into room temperature the secretion reached its peak Thereafter the secretion rate diminished but remained high as long as the body temperature was subnormal

Catecholamine levels in plasma were not significantly changed in man immediately following local exposure to cold (cold pressor test) (MANGER, WAKIM and BOLLMAN 1959), but, in hypothermic dogs, BROWN and COTTEN (1956) reported increased levels of circulating adrenaline and noradrenaline These results confirm the observations of the Japanese workers regarding the severity of cold stimuli for the secretion of adrenaline

The chromaffin cells in sections of the adrenal medulla after exposure to cold have also been studied CRAMER (1928) found a complete exhaustion of the fine granules which indicate the presence of adrenaline after exposure of mice to 5° C for a few hours Similarly, a noticeable reduction in the amount of secretory granular substance of the medullary cells of rats subjected to a temperature of 3° — 5° C for 2 hours was reported by LEVER (1955) The

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data of DAVIS, JOHNSTON BELL and CREMER (1960) indicate that about 50 per cent of the metabolic increase in acute exposure to cold is due to a centrally stimulated non shivering thermogenesis

CANNON QUERIDO, BRITTON and BRIGHT (1927) propounded the view that the secretion of adrenaline mediated part of the initial metabolic increase during cold exposure, and that this contribution of chemical regulation was masked by early shivering This opinion was not fully supported by later observations on the increase in oxygen consumption in normal and adrenal ectomized animals exposed to cold From the results of KAJIMA (1941), ONTI (1941), RING (1942), MORIN (1946, 1948) and COTTLE and CARLSON (1956), it can be concluded that the secretion from the adrenal medulla can account for not more than 10 per cent of the total increased metabolism produced by moderate cold stress The contribution of the adrenal medulla to the increased chemical thermogenesis (shivering excluded) was studied by SCHAEFFER (1946) and THIBAULT (1949) They found a decrease of 40 per cent in the metabolism of adrenalectomized rats exposed to cold It appears then that the adrenal medulla is not mainly responsible for the initial increased metabolism in animals exposed to cold The possible role of extra adrenal catecholamines in this respect has not been studied

The occurrence of non shivering thermogenesis in the cold acclimated animal is well demonstrated at least in the rat (SELLERS, SCOTT and THOMAS 1954, COTTLE and CARLSON 1956, HART, HEROUX and DEPOCAS 1956, DAVIS, JOHNSTON BELL and CREMER 1960) Such an increase in non shivering thermogenesis may be due either to a greater release of catecholamines or to a greater sensitivity of acclimated animals to these hormones or to both mechanisms We have already mentioned that an increased excretion of adrenaline and/or noradrenaline has been recently reported in rats chronically exposed to cold (COTTLE 1960 LEBLANC and NADEAU 1961) A greater sensitivity of cold acclimated rats to the calorigenic effect of adrenaline has been first observed by RING (1942) and confirmed later by other workers (HSIEH and CARLSON 1957, SWANSON 1957) HSIEH and CARLSON (1957) also reported a striking calorigenic action of noradrenaline in cold acclimated rats, which was confirmed by DEPOCAS (1960 a b)

Although the calorigenic effects of both adrenaline and noradrenaline are intensified in cold acclimated rats, acceptance of noradrenaline as the mediator of non shivering thermogenesis rests on the following observations (*cf* DEPOCAS 1961) Adrenal demedullation lowers the increase in oxygen consumption of the curarized cold acclimated rat at 6° C (COTTLE and CARLSON 1956) but does not abolish it as does previous injection of sympatholytic and ganglion blocking agents (HSIEH CARLSON and GRAY 1957) Noradrenaline is more effective than adrenaline in preventing the fall in oxygen consumption caused by hexamethonium in curarized cold acclimated rats (HSIEH, CARLSON and GRAY 1957) Cold acclimated rats show a greater sensitivity to injected

chromaffin reaction in the adrenals of rats exposed to 18° C was normal, but greatly reduced at 8° C (KAYSER 1939) and absent at 4° C (VINCENT 1925) However, recently FISHER, FISHER and FEDOR (1955) failed to reveal any alteration in the total chromaffin reaction of the medulla of rats subjected to hypothermic levels of cold Moreover, using a differential staining method, no change in the noradrenaline cells could be found

According to MORIN (1946) and SCHAEFFER (1946), not only the adrenal cortex showed hypertrophy as a result of prolonged exposure to cold, but also the adrenal medulla This view was not confirmed by recent histological studies (HEROUX 1960) which showed that the increase in weight of the adrenals on chronic exposure to cold was due exclusively to an increase in size of the fasciculata zone

In addition to these histological studies, direct estimations of catecholamines in the glands of animals exposed to cold have been performed HERMAN, CHATONNET and VIAL (1949) observed a marked decrease in the adrenaline content of adrenal glands in rats placed in a cold room at 2° C for periods of 15 minutes to 2 hours An extensive study of the catecholamine content of adrenals of rats on prolonged exposure to cold at 0° C and 10° C was made by DESMARAIS and DUGAL (1951) Their results indicate that after an initial depletion of adrenaline, and possibly noradrenaline, the amine content of the glands increases gradually over a period of about three weeks to figures which are higher than normal for noradrenaline at both temperatures while the adrenaline figures rise to normal values and show no significant change thereafter More recently, MOORE, CALVERT and BRODY (1961) reported an increased adrenaline and noradrenaline content of adrenal glands in female rats exposed to cold of 5° — 7° C for 3—5 weeks These workers also found a decreased amine content in heart but no change in brain

Less emphasis has been placed on the excretion of catecholamines on exposure to cold Only recently some reports have dealt with this aspect In man subjected to cold stress of 6 5° C for 1 hour, ARNETT and WATTS (1960) found a significant increase in the excretion of catecholamines, especially adrenaline In rats acclimated to cold, an increased excretion of noradrenaline (COTTLE 1960) and also adrenaline (LEBLANC and NADEAU 1961) was reported

It seems therefore established that there is an increased production and secretion of catecholamines in animals exposed to cold The next question to answer is to what extent are catecholamines involved in the chemical regulation of heat production Those experiments concerned with the increased metabolism on exposure and in acclimation to cold in relation to catecholamines will be briefly summarized

The presence of non-shivering thermogenesis in normal animals exposed to cold is still controversial On one hand, the results of COTTLE and CARLSON (1956) in fully curarized rats show that shivering is apparently the only means of additional heat production on exposure to cold On the other hand, recent

CHAPTER II

ESTIMATION OF CATECHOLAMINES IN URINE AND ORGANS OF RATS

GENERAL METHODS AND PHYSIOLOGICAL VARIATIONS

A. General methods

Selection and care of animals

All experiments were carried out on male rats of the Sprague-Dawley strain purchased from A B Anticimex (Stockholm) Rats were kept at least one week at room temperature (+ 22° C) before being used for experimentation Animals were carefully selected regarding their weight so that the differences between individuals in a group or between different groups were as small as possible Rats in the cold were kept in individual wire cages without bedding but in groups of six with bedding at room temperature They were daily fed a well balanced commercial brand of laboratory food and, unless otherwise stated, were allowed to eat and drink tap water *ad libitum* Cleaning of the cages was performed at regular intervals twice a week

In order to avoid twilight activity the rooms were artificially illuminated for a period of eight hours a day The different rooms were kept at the desired temperature ± 1° C However, the humidity in the cold rooms at + 3° C and — 7° C was 70 to 80 per cent compared to 50 to 60 per cent at room temperature

Experiments were arranged to be started at nine o'clock in the morning and urine was collected for the next 24 hours The colonic temperature was measured with a thermocouple whose tip was inserted to a depth of 5—6 cm and kept in place for 10 seconds The instrument was repeatedly calibrated against a mercury thermometer Colonic rather than rectal temperature was measured because of its greater constancy

Collection of urine

Except for rats weighing 100 g or less, at room temperature, urine was collected from individual rats in metabolism wire cages coated with plastic paint to prevent oxidation of catecholamines by metallic ions The cages were mounted on polythene funnels with a plug of pyrex wool in the neck which allowed filtration of urine but retained feces Urine specimens were collected in small polythene bottles and 1 N hydrochloric acid was added to urine so that the pH was maintained around 3 At the end of the collection period, usually 24 hours, care was taken to secure uniform collection of urine by slight pressure

noradrenaline than adrenaline (HSIEH and CARLSON 1957) There is a relationship between the calorigenic response and the dose of noradrenaline administered and also the time of exposure to cold (DEPOCAS 1960 a, b) A striking inverse relation exists between the increase in the metabolic response to noradrenaline infusion during acclimation to cold (DEPOCAS 1960 a, b) and the decrease in muscle electrical activity (HART, HEROUX and DEPOCAS 1956), suggesting a substitution of non shivering thermogenesis under the control of noradrenaline to the shivering heat production Neither cold exposure nor noradrenaline induces marked hyperglycemia as would be obtained by similar doses of adrenaline (HSIEH and CARLSON 1957)

It can therefore be concluded that, if the role of catecholamines in the immediate response to cold exposure is at the present not clear, a good deal of evidence supports the idea that the non shivering heat production, associated especially with acclimation to cold, is mediated through the release of noradrenaline, presumably from the adrenergic nerve endings

using an ordinary ultra violet filter (Omag Filter 404 a/3) inserted in the filter holder, which eliminated the high light scatter peak.

The methods showed a good degree of precision. Urine of six rats, weighing about 175 g, was collected for 24 hours, mixed together, divided in six equal portions and the aliquots carried through the complete procedure. The standard errors of the means for these six samples were 2.0 % for noradrenaline, 2.6 % for adrenaline and 3.1 % for dopamine. In a series of tests catecholamines were added to urine, in amounts [illegible]1 to 1 µg, and the recoveries were calculated after carrying the added amines through the entire procedure. The yields were on the average 80 % for noradrenaline, 72 % for adrenaline and 90 % for dopamine.

The catecholamine values reported in the present study were expressed as noradrenaline, adrenaline and dopamine hydrochloride, and they were not corrected for losses during the purification procedure.

B. Physiological variations in the excretion of catecholamines in rats

Variations with age and body weight

In an extensive study on the urinary excretion of noradrenaline and adrenaline in different age groups of humans, KARKI (1956) showed that the catecholamine excretion was related to the age and the body weight of the subjects. Similarly, we found such variations in the urinary output of catecholamines in rats. As seen in Fig. 1, there was a fairly good relationship between the amounts of amines excreted in urine and the body weight of animals. The correlation coefficients were + 0.80 for noradrenaline, + 0.81 for adrenaline and + 0.75 for dopamine. These relationships, however, were neither directly proportional nor similar for the three amines, as judged by the slopes of the curves. Therefore, when the results were expressed in µg per kilogram of body weight per 24 hours, large variations in the excretion values of catecholamines between the different groups were still present (Table I). This method of calculation presented the advantage that for adult rats of 175 g and more the values were more constant, although they still showed a gradual decrease with increasing weight. Since most of our experiments were performed on adult rats, the results reported in the present study were expressed in µg per kilogram of body weight per 24 hours.

Our adrenaline and noradrenaline excretion values in rats were higher than those previously reported by SCHAPIRO (1958) and PERMAN (1961) using a similar technique of estimation. In addition to such factors as the weight, sex and strain of rats which could partially account for the differences, it was suspected that the urine collection procedure was also of importance. These authors have pooled rats in a community metabolism cage while we used individual

of the abdomen in order to produce reflex urination Each metabolism cage was thoroughly rinsed with diluted acid and the washing waters added to urine specimens

Purification of urine and organ extracts

The procedure was essentially that used by EULER and LISHAJKO (1959, 1961 b) Each urine specimen was filtered and after addition of 0 5 g of the disodium salt of ethylenediamine tetraacetate (Titriplex, Merck) adjusted to pH 8 4 with 1 N sodium hydroxide under continuous magnetic stirring The catecholamines in urine were then immediately adsorbed on a column of about 0 75 g of alumina (British Drug Houses) previously washed and stirred with glass distilled water to remove air bubbles and let fine particles pass through the glass filter After passage of the urine specimens the alumina was carefully washed with glass distilled water Elution of catecholamines was performed with 2 × 2 5 ml of 0 3 N acetic acid Eluates collected in small bottles can be frozen and stored for a week before analysis without any appreciable loss of catecholamines

For organs, rats were killed by a blow on the head and organs immediately removed, weighed and extracted with trichloroacetic acid Except for adrenal glands, organs of three rats were pooled for extraction They were homogenized with an Ultra-Turrax apparatus in four to five volumes of 5 % trichloroacetic acid for heart and spleen and two to three volumes of 10 % trichloroacetic acid for liver and skeletal muscle The extraction was allowed to proceed for at least 30 minutes in the cold at + 4° C after homogenization Organ extracts were then filtered and treated as urine specimens Pairs of adrenals were extracted in 3 ml of 2 5 % trichloroacetic acid, filtered and appropriately diluted with 1 M acetate buffer pH 6 5 immediately before estimation of catecholamines In those cases where rats died in the cold, organs were removed as soon as possible after death and immediately frozen When organs from three animals were available they were pooled and extracted as previously described

Estimation of catecholamines

Noradrenaline and adrenaline in urine and organs were estimated according to the method of EULER and LISHAJKO (1959) The improved technique (EULER and LISHAJKO 1961 b) was used since December, 1960 No significant differences in the excretion values of noradrenaline and adrenaline were found when the two methods were compared

Dopamine was measured by the technique of CARLSSON and WALDECK (1958) and readings made at 330 mμ and 400 mμ with an Aminco-Bowman spectrophotofluorometer (uncorrected instrumental activating and fluorescent wavelengths) More consistent results, especially for dopamine in organs where the difference between the tissue blank and the sample was small, were obtained by

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Table I Urinary excretion of catecholamines in different weight groups of rats at room temperature (+ 22° C)

Weight in g	No of rats	Noradrenaline µg/kg/24 h Mean ± S E.	Adrenaline µg/kg/24 h Mean ± S E.	Dopamine µg/kg/24 h Mean ± S E
49	12	4.7 ± 0.25	0.00 ± 0.00	62 ± 2.8
75	12	4.8 ± 0.22	0.10 ± 0.02	63 ± 3.1
110	12	4.2 ± 0.21	0.25 ± 0.02	49 ± 2.5
174	12	3.6 ± 0.19	0.66 ± 0.04	32 ± 1.7
255	12	2.8 ± 0.16	0.60 ± 0.07	29 ± 1.6
375	12	2.7 ± 0.14	0.52 ± 0.03	30 ± 1.4
455	6	2.5 ± 0.20	0.48 ± 0.04	28 ± 1.9

Table II Individual and daily variations in the excretion of catecholamines in rats of 170—180 g at room temperature Urine collected from the same six animals for three consecutive days Standard deviations of the means expressed in per cent

Rat no	First day			Second day			Third day			Standard deviations		
	Nor	Adr	Dop.	Nor	Adr	Dop.	Nor	Adr	Dop.	Nor	Adr	Dop
	µg/kg/24 h			µg/kg/24 h			µg/kg/24 h			Per cent		
1	3.88	0.477	32.8	3.93	0.567	32.0	4.29	0.664	25.8	5.4	16.6	12.3
2	2.91	0.521	22.4	3.47	0.514	21.8	3.70	0.650	25.9	12.5	13.8	9.4
3	3.72	0.685	36.0	3.80	0.509	40.4	3.11	0.612	32.5	10.5	15.7	10.8
4	3.31	0.664	26.8	3.04	0.575	25.8	3.72	0.531	20.9	10.2	13.7	12.9
5	3.27	0.501	23.1	3.68	0.651	28.4	4.02	0.723	23.9	10.3	17.6	11.6
6	3.18	0.603	21.8	3.47	0.728	29.9	3.77	0.600	26.6	8.3	[illegible]	[illegible]
Mean	3.38	0.575	27.1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

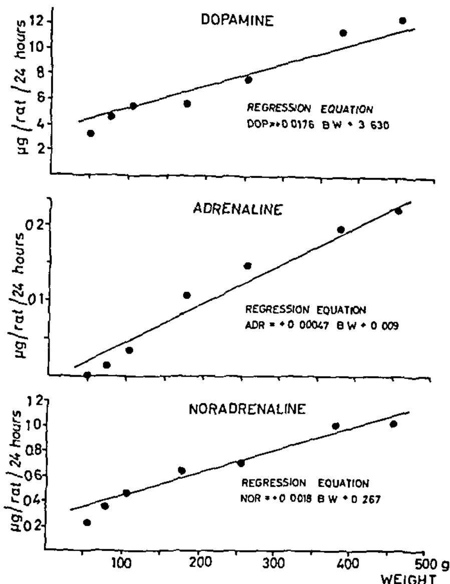


Fig 1 Correlation between the urinary excretion of catecholamines and the body weight of rats Curves calculated from the regression equations Twelve rats per group except the 455 g group (6 rats)

circular cages of 22 cm diameter We found in comparing the two procedures lower excretion values with the larger cages This was presumably due to a greater urine loss owing to the large evaporation surface and the difficulty in washing these cages after completion of the collection period

Individual and daily variations

When care was taken to secure uniform collection of urine by gentle pressure of the bladder and thoroughly washing of the cages individual variations between rats of the same weight were relatively small For the assays of nor

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Table II Individual and daily variations in the excretion of catecholamines in rats of 170—180 g at room temperature Urine collected from the same six animals for three consecutive days Standard deviations of the means expressed in per cent

Rat no	First day			Second day			Third day			Standard deviations		
	Nor	Adr	Dop	Nor	Adr	Dop	Nor	Adr	Dop	Nor	Adr	Dop
	µg/kg/24 h			µg/kg/24 h			µg/kg/24 h			Per cent		
1	3.88	0.477	32.8	3.93	0.567	32.0	4.29	0.664	25.8	5.4	16.6	12.3
2	2.91	0.521	22.4	3.47	0.514	21.8	3.70	0.650	25.9	12.5	13.8	9.4
3	3.72	0.685	36.0	3.80	0.509	40.4	3.11	0.612	32.5	10.5	15.7	10.8
4	3.31	0.664	26.8	3.04	0.575	25.8	3.72	0.531	20.9	10.2	13.7	12.9
5	3.27	0.501	23.1	3.68	0.651	28.4	4.02	0.723	23.9	10.3	17.6	11.6
6	3.18	0.603	21.8	3.47	0.728	29.9	3.77	0.600	26.6	8.3	10.9	15.7
Mean	3.38	0.575	27.1	3.57	0.592	29.7	3.77	0.632	25.9	9.5%	14.6%	12.1%
S D	12.0%	15.8%	22.1%	9.9%	7.0%	20.7%	11.3%	11.2%	11.0%			

adrenaline and adrenaline in urine of rats weighing 170—180 g, at room temperature, the standard errors of the means for six animals were usually between 5 and 8 per cent Even in rats exposed to cold, they were rarely exceeding 10 per cent. The dopamine values were more variable either between individuals or between groups of animals of about the same weight maintained at the same temperature The standard error of the mean for six rats was ordinarily between 10 and 15 per cent

In order to study the daily variations in the excretion of catecholamines, the urine of the same six rats was collected for a 24-hour period during three consecutive days and measured for the catecholamine content As shown in Table II, the standard deviations of the means of the three specimens from the same rat averaged 9.5 per cent for noradrenaline, 14.6 per cent for adrenaline

and 12 1 per cent for dopamine The standard deviations of the means when the six animals are considered as a group for each day show the variability in the dopamine excretion between individuals

The values presented in this table were expressed with three figures to show to what extent withdrawal of the third one could affect the results The error could theoretically be 10 %, but very seldom it exceeded 4—5 % The two-figure mode of expression was therefore adopted, which facilitated the reading of the tables without changing the interpretation of the results

Dietary variations

During the course of these experiments three different types of commercial laboratory food were used At one time we were supplied with a food which gave unusual excretion values for catecholamines Extraction of this food revealed that it contained a fluorescent substance which greatly interfered with the estimation of catecholamines The two other rations did not contain appreciable amounts of pre-formed fluorescent material Extracts from 10 g of food gave, after oxidation, a fluorescence which could influence the excretion values of catecholamines in rats of 170—180 g by at most 10 %.

On the other hand, while feeding of animals with either type of food did not affect the excretion of noradrenaline and adrenaline, large differences (25—35 %) *in the dopamine output were noted Since the substance measured in urine* gave the same spectral characteristics as a pure solution of dopamine, and since the possible interference from a pre-formed fluorescent material was ruled out by extraction of the food, two possibilities remained These commercial rations could contain substances which, metabolized in the organism, were excreted in urine as compounds which interfered with the estimation of dopamine Secondly, the dopamine formation, and consequently excretion, could be largely dependent on the type of diet The second possibility appeared to be more likely Rats fed with natural food (bread, cabbage) excreted as much dopamine as those maintained on one of the commercial ration Moreover, in starved rats the dopamine excretion decreased by 60 to 70 % the first day and continued to decrease on prolongation of starvation Finally, the dietary effect was more pronounced in those cases where the production of dopamine was large, as in very young rats or rats exposed to cold

Seasonal variations

A seasonal variation in the catecholamine content of various rat tissues has already been shown by Montagu (1959) The concentrations of total catechols, estimated by the ethylenediamine method, increased more extensively in the winter than could be accounted for by the minor changes of tissue weight which might occur In rats maintained at room temperature (+ 22 C) throughout the year, *the excretion of catecholamines in urine* also showed a seasonal pattern (Fig 2) The noradrenaline output was particularly affected,

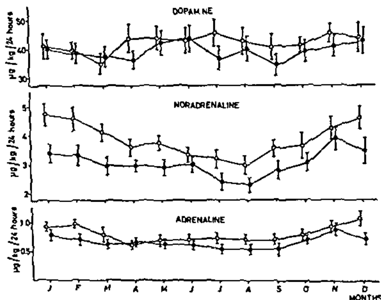


Fig 2 Seasonal variations in the catecholamine excretion of rats weighing 170—180 g (○) and 250—350 g (●) maintained throughout the year at + 22° C. Each point represents the mean of six animals Standard deviations indicated by vertical lines.

being significantly lower during the summer months than during the winter months The adrenaline excretion also decreased during the summer time, but seasons had no influence on the dopamine output The twilight activity was minimized by keeping the room artificially illuminated all year long for an 8 hour period a day

We have also noted a lower catecholamine content, especially noradrenaline, in adrenal glands of rats during the summer time We do not have enough values for other organs to allow any definite conclusion, although the general trend was in the same direction The variations of the concentrations of catecholamines in urine and organs might explain some seasonal changes of metabolism which have been reported in rats kept in the laboratory throughout the year (BENEDICT and MACLEOD 1929, SHERWOOD 1936)

Summary

The methods used for the estimation of catecholamines in urine and organs showed a good degree of precision Individual and daily variations in the catecholamine excretion of rats at room temperature were relatively small when care was taken to secure uniform collection of urine Physiological factors, such as the weight of animals, the type of diet and the seasons, influenced the urinary output of catecholamines

CHAPTER III

EFFECT OF EXPOSURE TO COLD ON THE PRODUCTION AND RELEASE OF CATECHOLAMINES

A number of studies regarding the catecholamine secretion, especially adrenaline, in response to cold stress have been performed, as reviewed in Chapter I It is obvious, however, that measurement of the catecholamine production and release in prolonged exposure to cold has not so far been achieved The actual catecholamine content of adrenal glands has been estimated at various times during acute and prolonged cold exposure (HERMAN, CHATONNET and VIAL 1949, DESMARAIS and DUGAL 1951, MOORE, CALVERT and BRODY 1961) However, the difficulty in drawing conclusions as regard to the amine production from the actual content of the glands is illustrated by recent experiments on resynthesis during insulin hypoglycemia (BYGDEMAN, EULER and HOKFELT 1960) There might be a considerable release without any marked reduction in the content of the glands in many cases, but also when the glands are largely depleted of their amines At the present time, the only available method to follow the catecholamine production over a long period of time is by measuring the output in urine in conjunction with estimations of the amine content of the glands in parallel experiments

The results of the Japanese workers (WADA, SEO and ABE 1935, SAITO 1928) have pointed to the intensity of cold stimuli as an important factor for the secretion of adrenaline from the adrenal glands Acute experiments on the production and release of catecholamines during exposure to different temperatures were therefore pursued Whether or not other factors such as the body weight of animals and the seasons, which have been shown to affect the catecholamine output in normal rats (*cf* Chapter II), could influence the catecholamine response to cold exposure was also investigated

Methods

Male rats were used throughout these experiments and carefully selected so that they could be classified *into four categories according to their weight* young rats of 100—110 g young adult rats of 170—180 g adult rats of 250—350 g and old rats of 450—
[illegible] had free access
° C
[illegible]

at least in rats at room temperature

In many experiments a large number of rats were exposed to cold at the same time and, unless otherwise specified, the results represent the means of six animals randomly assigned for the estimation of catecholamines in urine and organs Experimental groups were always compared with controls maintained at room temperature (+ 22 C

This control group although of the same age, was of a heavier weight when chronic experiments were performed

Three rats were pooled for the estimation of catecholamines in organs except adrenal glands The calculation of the adrenaline resynthesis was based on the following principle (*cf* BYGDEMAN, EULER and HOKFELT 1960) From the excretion values of adrenaline in urine, it is possible, by referring to the excretion after giving known amounts of this hormone, to calculate the secretion of adrenaline, assuming that the same relationships exist between the amounts excreted after injection and those excreted after secretion into the suprarenal veins Then from the rate of secretion and the actual content of the glands the rate of resynthesis can be computed This method gives an idea of the resynthesis of adrenaline in the adrenal glands since it is generally accepted that most of the adrenaline excreted in urine comes from the suprarenals (*cf* EULER 1956), but it does not allow any conclusion on the resynthesis which can occur in extra adrenal chromaffin cells These experiments were always made in parallel, that means that the same animals were used for the collection of urine and killed afterwards for the catechol amine estimation of the glands

In recovery experiments six rats maintained at room temperature six rats which have been in the cold at + 3° C for six days and six rats acclimated to + 3° C for one month were used Three animals in each group were injected [illegible] hydrochloride and the three others [illegible] catecholamines excreted in urine du[illegible] those excreted by the same rats the [illegible] given at a dose of 100 μg rat the difference between the excretion values of adrenaline or noradrenaline for the two days represented the percentage of the injected dose excreted as free catecholamin

The effect of diuresis on the excretion of catecholamines was studied in six rats weighing on an average 250 g and maintained at room temperature The first day rats were allowed to drink tap water *ad libitum* the second day saline (1 % NaCl) was substituted to water, the third day tap water was restored and the fourth day rats had no access to water

Results

Catecholamine excretion on exposure to cold[1]

The excretion of noradrenaline and adrenaline in rats of 170—180 g exposed to a temperature of + 3° C for one month is seen in *Fig* 3 The noradrenaline output was nearly maximal during the first 24 hours and remained high even after one month of exposure, although it showed a slow and steady decline with time In some experiments where the collection of urine was fractionated in two 12 hour periods during the first day of exposure to cold, the noradrenaline excretion was found to be as high during the first 12 hour period as during the second one, indicating an immediate maximal response in the noradrenaline excretion On the contrary, the adrenaline output gradually increased to a maximum in about one week in the cold and decreased thereafter more rapidly than noradrenaline However, it was still higher than in the control group after one month in the cold at + 3° C

[1] A preliminary report of this work has been published (LEDUC 1961)



Fig 3 *Urinary excretion of adrenaline* (●) and noradrenaline (○) *in rats* (170—180 g) at + 3° C (—) *and* + 22° C (-) *Each point represents the mean of six individual rats*

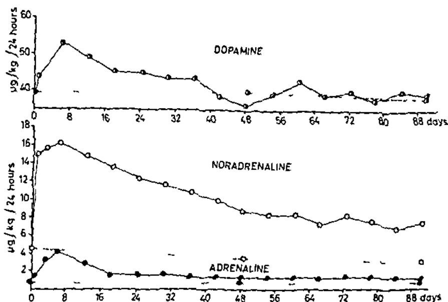


Fig 4 *Urinary excretion of adrenaline* (●), *noradrenaline* ( ) *and dopamine* (◗) *in rats* (170—180 g) at + 3° C (—) and + 22° C ( ) Each point represents the mean of six individual rats

Regarding the excretion of catecholamines in rats of *170—180* g exposed to a temperature of + 3° C, we should emphasize here some points In all groups studied, the typical curves for adrenaline and noradrenaline shown in Fig 3 were observed The noradrenaline excretion was always nearly maximal during *the first 24 hours and later on slowly decreased with time In some cases the* slope of the curve was more accentuated (Fig 4) In long-term experiments it has appeared that the decline in the noradrenaline curve was more pronounced

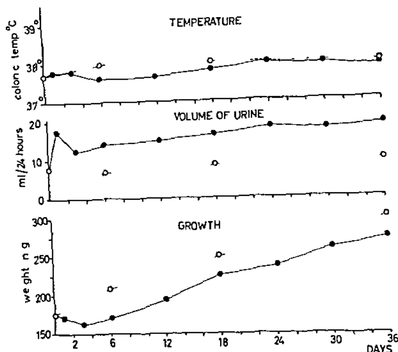


Fig 5 Colonic temperature diuresis and growth of rats (170—180 g) exposed to cold of + 3° C (—) or maintained at + 22° C ( ) Each point represents the mean of six rats

during the second month of exposure Thereafter the noradrenaline excretion remained at about the same level as long as rats were kept in the cold (up to 6 months) This steady level was at least 2 5 to 3 5 times as high as the values for the control group, as seen in Fig 4 For adrenaline, there was always a peak excretion after one week in the cold and a more rapid decrease thereafter, but in any case to the normal level Even when rats were kept for months in the cold (Fig 4) the adrenaline excretion was still 50 to 100 per cent higher than in the control group The excretion of dopamine was more variable, although in rats of 170—180 g exposed to + 3° C there was always a peak in the output of this amine after about one week of exposure with a return to normal values within a month The dopamine excretion at its maximum was usually 30 to 50 per cent above the values for rats maintained at room temperature (Fig 4) The absolute excretion values of catecholamines in rats exposed to cold varied according to such factors as the body weight of animals and the seasons but the general trend was always in the same direction

Table III Excretion of catecholamines in relation to the rate of urine flow. Experiments conducted on four consecutive days with the same six rats weighing about 250 g

	Diuresis ml/24 h	Noradrenaline µg/kg/24 h Mean ± S.E.	Adrenaline µg/kg/24 h Mean ± S.E.	Dopamine µg/kg/24 h Mean ± S.E.
Water *ad libitum*	6.9	2.4 ± 0.18	0.55 ± 0.03	22 ± 2.4
1 % NaCl	43.5	3.0 ± 0.21	0.62 ± 0.04	28 ± 2.8
Water *ad libitum*	8.3	2.5 ± 0.19	0.68 ± 0.03	24 ± 2.9
No water	5.0	2.9 ± 0.15	0.86 ± 0.04	23 ± 3.1

At all times the colonic temperature of rats exposed to + 3° C was normal (Fig. 5). Even when the temperature was measured every three hours, except the diurnal variations also present in the control group at room temperature and relatively small, no changes were noted. The high excretion of adrenaline during the first week of exposure was not associated with any degree of hypothermia.

A two-fold increase in diuresis was observed in rats exposed to cold (Fig. 5). Since variant results were reported on the effect of diuresis on the excretion of catecholamines in rats (PITKANEN 1956, PERMAN 1961), this point was further investigated. In rats at room temperature diuresis by itself had little effect, if any, on the excretion of catecholamines (Table III). Increasing diuresis by 5 to 6 times did not significantly change the excretion of noradrenaline, adrenaline and dopamine. Restriction of water, on the other hand, significantly increased the adrenaline output ($p < 0.01$).

During the first week of exposure to cold growth was delayed (Fig. 5) and some animals lost as much as 10 to 15 grams. Thereafter growth proceeded regularly but at a slower rate than in the control group. The weight loss during the first week of exposure to cold may explain the slightly higher excretion values of noradrenaline the sixth day than the first day and lower by 5 to 10 per cent the excretion values of adrenaline and dopamine.

The increased excretion of catecholamines in rats exposed to cold might partially be due to an impairment in the destruction of these amines in the cold. Similarly, the decline in the curves might be due to an activation of the degradation processes. Recovery experiments after injection of catecholamines in rats exposed to cold for one week, when the excretion values were the highest and one month, when the decline in the noradrenaline curve was more pronounced, showed that there was neither an inhibition nor an activation of the destruction of catecholamines in rats exposed to cold (Table IV). It is important to note that the excretion of adrenaline was not changed after injection of noradrenaline and *vice versa*.

Table IV Adrenaline and noradrenaline recovered in urine after subcutaneous injection in normal and cold-exposed rats Results express the differences in catecholamines between the day of injection and the day before for the same three animals

Groups	No of rats	Noradrenaline	Adrenaline	Dopamine
		µg/rat/24 hours		
Injection of *l-adrenaline* 100 µg/rat s.c				
Room temp +22° C	3	+0 09	+3 4 (±0 20)	+0.27
6 days at +3° C	3	+0 02	+2 6 (±0 32)	−0 18
30 days at +3° C	3	+0 15	+2 5 (±0 33)	−0 48
Injection of *l-noradrenaline* 100 µg/rat s.c				
Room temp +22° C	3	+2 8 (±0 10)	−0 01	−1 50
6 days at +3° C	3	+2 4 (±0 26)	−0 04	+0 44
30 days at +3° C	3	+2 7 (±0 25)	+0 02	+0 35

Relation between the noradrenaline excretion and the time of exposure to cold

The noradrenaline excretion curve declined with the time of exposure to cold, and one could argue that this effect was due to the increase in the body weight of animals Indeed, when results are expressed on a weight basis, there is a decline in the noradrenaline excretion with increasing weight (*cf* Chapter II)

It became evident, however, that the weight factor was partly responsible for the decline in the noradrenaline curve, but not exclusively In experiments of one month duration the weight factor was greatly responsible for this effect In some cases the relative decrease in the excretion of noradrenaline in rats exposed to cold was of about the same magnitude as the relative decrease in the control group at room temperature However, as mentioned before, the decline in the curve was more pronounced during the second month, and in long-term experiments there was a significant difference between the control and the experimental groups For instance, in two groups of rats which have been in the cold for three months the noradrenaline excretion decreased by 54 and 58 per cent while in their respective control groups the relative decreases were 31 and 36 per cent The same difference of 20 to 25 per cent was noted in

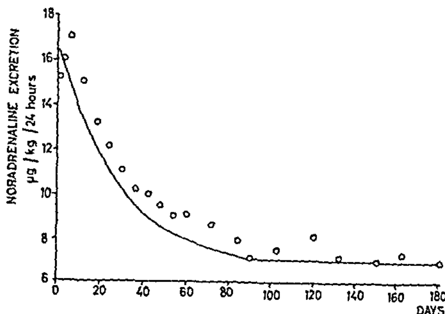


Fig 6 Urinary excretion of noradrenaline in rats (170—180 g) exposed to + 3° C in relation to the time of exposure Curve calculated from the regression equation $Y = 6\ 80 + 9\ 82 \exp - 0\ 037\ X$, where Y is the noradrenaline excretion and X the time in days Each point represents the mean of 12 rats

rats maintained in the cold for 6 months Also, about the same difference was observed in comparing the excretion of noradrenaline in rats maintained in the cold for 3 or 6 months with a control group of the same weight exposed to cold for the first time The latter group excreted 25 to 30 per cent more noradrenaline than cold-acclimated rats (*cf* Chapter IV, Fig 14) Moreover, this slow and steady decline in the noradrenaline curve was seen in old rats (250—350 g) exposed to cold while the noradrenaline excretion of the controls at room temperature was fairly constant Finally, this decline in the curve was not observed in cold-acclimated rats re-exposed to cold (*cf* Chapter IV, Fig 14)

It was therefore justified to correlate the excretion of noradrenaline to cold acclimation The data stongly suggested an asymtotic relationship of the form $Y = a + b \exp - c X$, where Y is the noradrenaline and X the time of exposure to cold Maximum likelihood estimates of the parameters were calculated by a method described by Stevens (1959)[1] The values of the parameters were $a = 6\ 80$, $b = 9\ 82$ and $c = 0\ 037$ The curve shown in Fig 6 was calculated from the equation $Y = 6\ 80 + 9\ 82\ \exp - 0\ 037\ X$ The observed values showed as good a fit as could be expected with those calculated from the regression equation (probability 97 %) The period required for half-maximal decrease in the noradrenaline excretion was approximately 20 days

[1] The statistical analysis of these data was kindly made by Mr J St Pierre

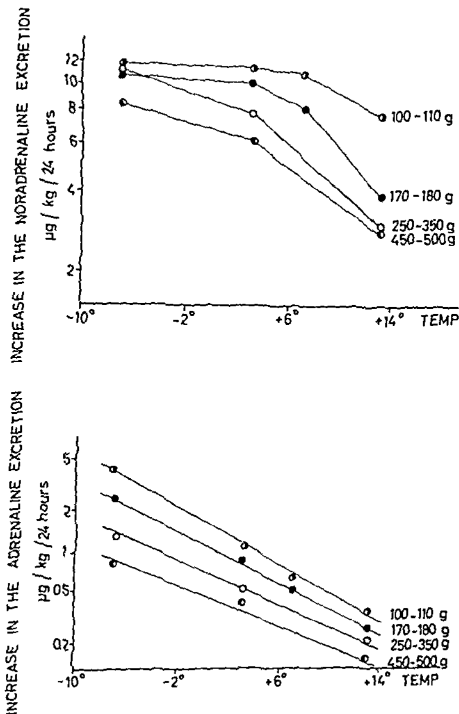
Table V Seasonal variations in the catecholamine excretion of rats (170—180 g) exposed to cold (+ 3° C) Each value represents the mean of six rats

Days in the cold +3°C	January February			April May			July August			November December		
	Nor	Adr	Dop	Nor	Adr	Dop	Nor	Adr	Dop	Nor	Adr	Dop
	μg/kg/24 h Mean ± S E.			μg/kg/24 h Mean ± S E.			μg/kg/24 h Mean ± S E.			μg/kg/24 h Mean ± S E.		
0	4 7 ±0 16	0 89 ±0 03	39 ±1 9	3 4 ±0 17	0 68 ±0 04	42 ±2 7	2 9 ±0 15	0 72 ±0 04	42 ±2 9	4 0 ±0.21	0 86 ±0 03	40 ±2 2
1	15 4 ±0 97	1 42 ±0 04	49 ±2 7	14.2 ±1 05	1 71 ±0 06	44 ±2 1	11.3 ±0 86	1.87 ±0 08	41 ±3 1	16.6 ±0 96	2.25 ±0 10	53 ±3 8
3	16 0 ±1 05	3.21 ±0 06	56 ±3 2	11 8 ±0 80	2 44 ±0 07	47 ±2 4	13 0 ±0 68	2 84 ±0 11	57 ±3 8	15 4 ±0 94	3 82 ±0 12	61 ±3 9
6	17 4 ±1 10	4 17 ±0 16	61 ±3.2	12 7 ±0 92	3 29 ±0 13	51 ±2 8	12 5 ±0 76	3 49 ±0 11	61 ±3 7	15.2 ±0.86	5 01 ±0.20	54 ±3.2
12	14 8 ±0 82	2 94 ±0 08	50 ±3 9	12 0 ±0 76	1 78 ±0 08	45 ±2 6	12 7 ±0 93	1 92 ±0 07	44 ±2 7	14.2 ±0 60	2 44 ±0 08	44 ±2 7
18	13 2 ±0 64	1 64 ±0 04	53 ±2.2	11 0 ±0 63	1 35 ±0 05	48 ±3.2	12 4 ±0 65	1.55 ±0 07	38 ±2 9	13 4 ±0 71	1 97 ±0 11	40 ±3 1
24	12 0 ±0 82	1 54 ±0 03	45 ±2.2	9 6 ±0 48	1.30 ±0 06	44 ±1 9	11 3 ±0 69	1.25 ±0 05	38 ±2 8	12.2 ±0 62	1 40 ±0 08	37 ±3 8
30	10 3 ±0 64	1 60 ±0 05	48 ±2 7	9 8 ±0 64	1 13 ±0 04	41 ±2 4	10 8 ±0 62	1.23 ±0 05	40 ±2.2	10.2 ±0 78	1.24 ±0 04	38 ±1 9

Variations in the excretion of catecholamines

The catecholamine excretion in rats at room temperature showed seasonal variations (*cf* Chapter II) Similarly, seasons affected the catecholamine excretion of rats exposed to cold Table V shows the excretion of noradrenaline, adrenaline and dopamine in four groups of rats exposed to + 3° C for one month at different periods of the year The absolute values for noradrenaline and adrenaline were higher during the winter time than during the summer time However, when the relative increases over the respective control values were calculated, it appeared that there was no difference between the four groups This suggested that seasons did not qualitatively influence the catecholamine response of rats exposed to cold Support for this opinion was given by the lack of influence of seasons on the dopamine excretion in rats exposed to cold as well as in rats maintained at room temperature throughout the year (*cf* Chapter II)

The excretion of catecholamines in rats exposed to cold was also dependent on the body weight of animals and the temperature of exposure The adrenaline output was related to these factors but not directly proportional either to the



F... 7. Correlation between the increase in the noradrenaline (upper graph) and adrenaline

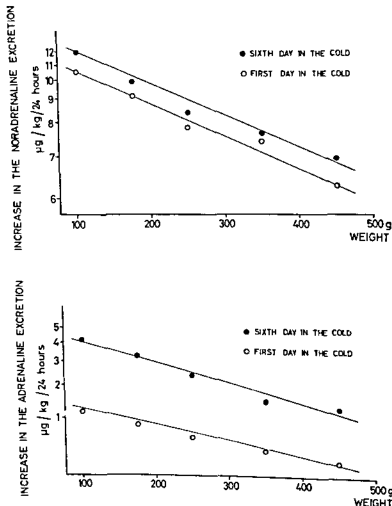


Fig 8 Correlation between the increase in the noradrenaline (upper graph) and adrenaline (lower graph) excretion and the body weight of rats exposed to + 3° C. Twelve rats per group

body weight of rats or the temperature of exposure For instance, the increase in the adrenaline excretion between + 22° C and + 3° C was less than the increase between + 3° C and — 7° C, and less for rats of 175 to 250 g than for those of 100 to 175 g These data suggested a logarithmic relationship between these variables In Fig 7 we have plotted on a logarithmic scale the increase in the adrenaline excretion the first day in the cold against the temperature of exposure for four groups of rats of different body weights In Fig 8 the same type of relation has been established between the increase in the adrenaline

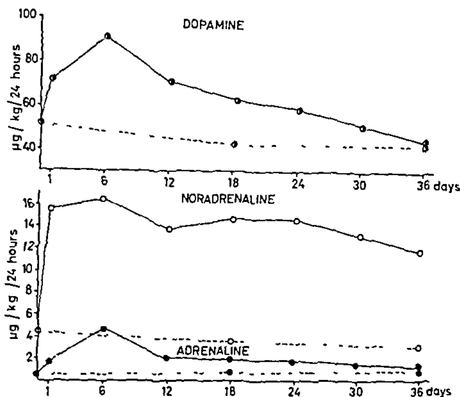


Fig 9 Urinary excretion of adrenaline (●), noradrenaline (○) and dopamine (◑) in young rats (100—110 g) at + 3° C (—) and + 22° C ( ) Each point represents the mean of six individual rats at + 3° C and of six groups of 2 rats at + 22° C

excretion and the body weight of rats in chronic experiments at + 3° C It is evident from these curves that the adrenaline excretion is linearly related, at least under these experimental conditions, to the body weight of animals and the temperature of exposure

Similar curves have been drawn for noradrenaline A linear relationship between the increase in the noradrenaline output and the body weight of animals in chronic experiments at + 3° C was still present (Fig 8), although the differences between the groups were not very large On the contrary, in experiments at various temperatures of exposure the relation was no longer linear (Fig 7) but in this case the noradrenaline excretion arrived at a plateau level when the environmental temperature was lowered This limit was reached more or less rapidly according to the body weight of animals

The excretion of dopamine was also related to the body weight of animals While rats of 100—110 g showed an increase in the dopamine output of about 70 to 80 % the sixth day of exposure to cold at + 3° C (cf Fig 9), rats of 170—180 g increased by only 30 to 50 % (cf Fig 4) and rats of 250 to 500 g sometimes showed an increase of 20 to 40 % but in other cases no significant changes In acute experiments at different temperatures such a relation as for adrenaline

Table VI Catecholamine content of adrenal glands in rats exposed to cold at + 3° C Each value represents the mean of nine individual rats

Days	Controls + 22° C		Cold-exposed + 3° C	
	Noradrenaline	Adrenaline	Noradrenaline	Adrenaline
	µg/kg b. wt. ± S E.		µg/kg b wt. ± S E.	
1	22.2 ± 1.2	122 ± 4 7	20 7 ± 1.2	95 ± 3.9
6	21 8 ± 1 1	119 ± 4.2	27 3 ± 1.3	137 ± 5 1
18	23 6 ± 1 1	125 ± 3 7	21.3 ± 1.2	136 ± 4.2
36	22 3 ± 1 1	130 ± 4.2	21 1 ± 1 1	141 ± 4.2
60	19.2 ± 1.2	136 ± 3 9	19 4 ± 1.2	151 ± 3 7
90	23 6 ± 1.3	147 ± 4 5	24 6 ± 1 4	163 ± 4 1
180	24 8 ± 1 3	151 ± 4 9	26 3 ± 1.3	175 ± 4 9

Table VII Adrenaline secretion and resynthesis in rats exposed to cold at + 3° C There were six animals at each time

Days in the cold + 3° C	Differences in the glands µg/kg b wt.	Excretion in urine µg/kg/24 h	Adrenaline	
			Secretion µg kg/24 h	Resynthesis µg/kg 24 h
Controls + 22 C, 175 g	0	0 86	27	27
1	− 19	1 48	46	27
2	0	2 69	82	82
4	+ 14	4 30	136	150
6	+ 16	6 02	190	206
8	+ 24	5 73	181	205
10	+ 22	3 60	114	136
12	+ 18	2 74	87	105
15	+ 12	2 47	78	90
18	+ 10	2 33	73	83
24	+ 15	1 70	54	69
30	+ 8	1.23	39	47
36	+ 9	1 15	36	45
Controls + 22 C, 290 g	0	0 70	22	22

and noradrenaline could not be established For rats of a given body weight the increase in the dopamine output was approximately the same at + 13° C, + 3° C or — 7° C

Catecholamine content of organs

In rats of 170—180 g exposed to + 3° C there was a significant depletion in the adrenaline content of adrenal glands after 24 hours in the cold (Tables VI and VII) By the second day, however, the adrenaline concentration was normal

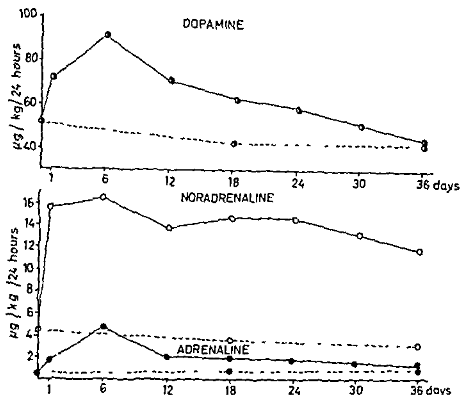


Fig 9 Urinary excretion of adrenaline (●), noradrenaline (○) and dopamine (◑) in young rats (100—110 g) at + 3° C (—) and + 22° C ( ) Each point represents the mean of six individual rats at + 3° C and of six groups of 2 rats at + 22° C

excretion and the body weight of rats in chronic experiments at + 3° C It is evident from these curves that the adrenaline excretion is linearly related, at least under these experimental conditions, to the body weight of animals and the temperature of exposure

Similar curves have been drawn for noradrenaline A linear relationship between the increase in the noradrenaline output and the body weight of animals in chronic experiments at + 3° C was still present (Fig 8), although the differences between the groups were not very large On the contrary, in experiments at various temperatures of exposure the relation was no longer linear (Fig 7) but in this case the noradrenaline excretion arrived at a plateau level when the environmental temperature was lowered This limit was reached more or less rapidly according to the body weight of animals

The excretion of dopamine was also related to the body weight of animals While rats of 100—110 g showed an increase in the dopamine output of about 70 to 80 % the sixth day of exposure to cold at + 3° C (cf Fig 9), rats of 170—180 g increased by only 30 to 50 % (cf Fig 4) and rats of 250 to 500 g sometimes showed an increase of 20 to 40 % but in other cases no significant changes In acute experiments at different temperatures such a relation as for adrenaline

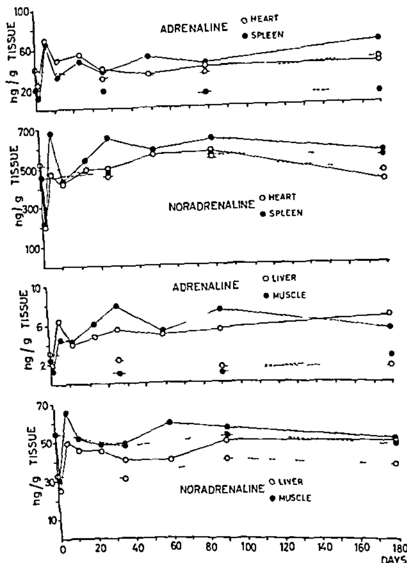


Fig 11 Catecholamine content of organs in rats at + 3° C (—) and + 22° C ( ) There were three groups of three rats at each time

linear in the dose range of 25 to 250 μg/kg with an average of 3 2 % of the injected dose excreted in urine in 24 hours (Fig 10) This constant of 3 2 was used in calculating the secretion of adrenaline from the actually observed excretion values As seen in Table VII, the resynthesis was not accelerated during the first 24 hours in the cold By the second day the resynthesis increased

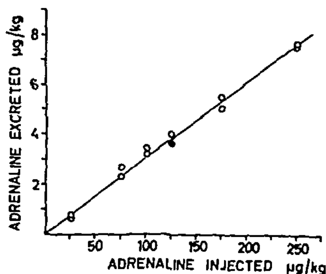


Fig 10 Correlation between injected amounts of adrenaline and quantities recovered in urine as free amine in 24 hours

Table VIII Catecholamine content of adrenal glands and adrenaline secretion and resynthesis in relation to the temperature of exposure and the body weight of rats in acute experiments (24 hours) There were six animals in each group

Weight	Temp	Adrenal glands		Adrenaline in urine	Adrenaline	
		Nor-adrenaline	Adrenaline		Secretion	Resynthesis
		μg/kg b wt ± S E		μg/kg/24 h	μg/kg/24 h	
100—110 g	+ 22° C	19 2 ± 0 6	124 ± 4 1	0 52 ± 0 06	16	16
	+ 3° C	16 6 ± 1 4	93 ± 5 1	1 72 ± 0 09	54	23
	− 7° C	14 5 ± 1 6	85 ± 2 5	4 35 ± 0 24	137	96
170—180 g	+ 22° C	20 8 ± 1 4	120 ± 3 8	0 70 ± 0 03	22	22
	+ 3° C	20 7 ± 1 0	100 ± 3 9	1 45 ± 0 09	46	26
	− 7° C	18 2 ± 1 6	97 ± 3 2	3 24 ± 0 25	102	79
300—350 g	+ 22° C	16 0 ± 1 4	127 ± 4 1	0 63 ± 0 04	20	20
	+ 3° C	16 1 ± 1 1	128 ± 4 1	1 15 ± 0 09	36	37
	− 7° C	14 2 ± 1 0	136 ± 4 1	1 98 ± 0 13	63	72
450—500 g	+ 22 C	18 7 ± 1 2	134 ± 5 4	0 45 ± 0 03	14	14
	+ 3° C	17 7 ± 1 3	125 ± 4 2	0 93 ± 0 04	30	21
	− 7 C	16 3 ± 1 4	142 ± 5 3	1 27 ± 0 13	40	48

and later on increased over the control values as long as rats were kept in the cold (up to 6 months) The noradrenaline figures did not show any significant change except a slight increase after one week in the cold

For the calculation of the rate of resynthesis of adrenaline, the correlation between the amounts of adrenaline injected and the quantities recovered in urine in 24 hours was first established In our hands the relationship was

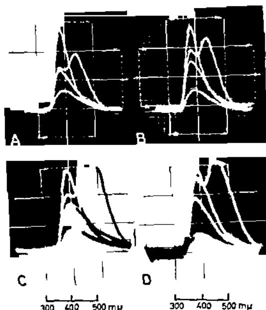


Fig 13 Fluorescent spectra of dopamine in the spleen (A) liver (C) and skeletal muscle (D) of rats exposed to + 3° C for one month and in the spleen (B) of rats maintained at room temperature From top to bottom curves represent dopamine standard, tissue sample tissue blank and reagent blank. Activating wavelength, 330 mμ

concentrations in spleen, liver and skeletal muscle remained high The adrenaline variations were probably significant, but we must add that these estimations were done on only three groups of three rats and that the adrenaline content of organs usually showed large variations

Dopamine was normally present in the spleen of rats On exposure to cold it completely disappeared from this organ and, except the first day, no dopamine could be detected in the spleen of cold exposed rats even after six months in the cold (Fig 12) On the contrary, in liver and skeletal muscle, in which organs dopamine was normally absent or in very low concentrations, rather large amounts were found in rats exposed to cold (Fig 12) There was a definite peak in the dopamine content of these tissues after one month in the cold At no time was dopamine found in heart Fig 13 shows pictures of the fluorescence spectrum of dopamine in the spleen, liver and skeletal muscle of rats at room temperature and in the cold at + 3° C It can be seen that the differences between the samples and the tissue blanks were not very large

Discussion

From the observations of Hsieh, Carlson and Gray (1957) that the sympathetic nervous system plays an important part in the mediation of chemical regulation of heat production, and that noradrenaline can prevent the fall in oxygen consumption caused by hexamethonium in curarized cold acclimated

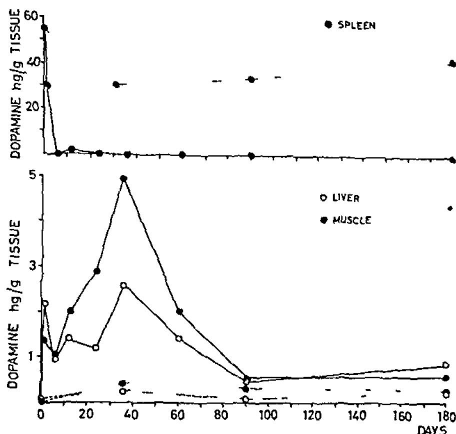


Fig 12 Dopamine content of the spleen liver and skeletal muscle of rats at room temperature (–) and in the cold at + 3 C (—) There were three groups of three rats at each time

rapidly reaching a maximum after one week in the cold It decreased thereafter but was still greater than in the control group after one month of exposure to cold The adrenaline resynthesis measured in long term experiments was found to be higher than normal even after 6 months in the cold

The initial depletion in the adrenaline content of the glands and the secretion and resynthesis of adrenaline in rats exposed to cold varied according to the body weight of animals and the temperature of exposure (Table VIII) While in young rats there was a depletion in the adrenaline content of the glands which was more marked at — 7° C than + 3° C in old rats there was rather an increase in the adrenaline content which was greater at — 7° C than + 3° C The resynthesis of adrenaline was more accelerated in young rats than old ones and at — 7° C than + 3° C

In all other organs studied (heart, spleen liver and skeletal muscle) there was a depletion (30—50 %) in both amines after 24 hours in the cold at + 3 C (Fig 11) On the sixth day of exposure to cold noradrenaline and adrenaline in all organs were in higher concentrations than normally Thereafter the noradrenaline concentrations returned to the normal values while the adrenaline

noradrenaline infusion (DEPOCAS 1960 a, b) Plateau values are reached at a later time, which suggests that our curve represents the sum of two or more components in the noradrenaline excretion which are not related either to its metabolic action or to the acclimation process Pertinent to the first possibility is the activation of the adrenergic vasomotor nerves to the skin and other regions when the organism is exposed to cold (CANNON, QUERIDO, BRITTON and BRIGHT 1927, SATOW 1937, WADA and FUZII 1940) The effect of noradrenaline released by cold may therefore be assigned to its vasoconstrictor action as well as its stimulating effect on metabolism, and we do not know if cold acclimation modifies the former effect The second explanation is supported by recent observations of DAVIS, JOHNSTON, BELL and CREMER (1960) which indicate that the cold induced oxygen consumption in rats represents the algebraic sum of three mechanisms a peripherally stimulated oxygen consumption due to shivering and abolished by curare, which gradually disappears with time and is replaced by a non shivering peripherally stimulated oxygen consumption unaffected by curare and possibly due to increase in tissue metabolism, and finally

unaffected

be influenc

suggesting that the sympathetic nervous system is also involved in this mechanism Therefore, part of the noradrenaline released by cold can be utilized for this effect Since it is not affected by acclimation, its contribution to the total noradrenaline output can partly mask the decline in the curve These two additional factors for the release of noradrenaline on exposure to cold can explain the immediate maximal response in the noradrenaline secretion which otherwise, in view of the weak calorigenic action of this hormone at that time (*cf* DEPOCAS 1960 a, b), may appear to be a wasteful aspect of cold defence The existence of a true chemical regulation in normal rats exposed to cold will also help to understand some observations hardly explainable if one considers shivering as the only means of heat production in this case

Regarding the role of adrenaline in the catecholamine response to cold, our results favor the hypothesis that this hormone acts as a second line of defense which need not be called on for great activity unless the noradrenaline mechanism becomes saturated This suggestion is based on the finding that the noradrenaline synthesis and/or secretion is *readily limited* On expos

increase in the adrenaline excretion under the same conditions does not mean that there is no limit for the adrenaline synthesis and/or secretion but probably *that, in these experiments*, it has not been attained The difference between adrenaline and noradrenaline, besides being due to different rate of resynthesis, may also reside in the reserves of the organism which are possibly larger for adrenaline (adrenal glands) than nor-

rats, it can be deduced that the chemical regulation of heat production is mediated through the release of noradrenaline from the sympathetic nerve endings The large and sustained excretion of noradrenaline in rats chronically exposed to cold strongly supports this idea of noradrenaline as the mediator of non shivering thermogenesis

The adrenaline excretion pattern with a definite peak after one week in the cold and a rapid decrease thereafter speaks against the assumption of adrenaline as the main factor in the chemical regulation of heat production (CANNON, QUERIDO, BRITTON and BRIGHT 1927, MORIN 1946, 1948) Furthermore, cold acclimation does no induce hyperglycemia as would be expected if adrenaline was the mediator (HSIEH and CARLSON 1957) Adrenaline is also much less effective than noradrenaline in preventing the fall in oxygen consumption caused by hexamethonium (HSIEH, CARLSON and GRAY 1957)

The main change brought about by acclimation to cold appears to be an alteration in the response of the tissues to the calorigenic effect of noradrenaline (HSIEH and CARLSON 1957, DEPOCAS 1960 a, b), which increases the ability of cold-acclimated rats to produced heat by chemical thermogenesis This in creased sensitivity to noradrenaline may be related to the decline in the noradrenaline excretion with the time of exposure to cold

DEPOCAS (1960 a, b) has shown that the full development of the increased sensitivity to the calorigenic effect of noradrenaline required about one month in rats exposed to cold at + 6° C The fairly good inverse resemblance between Depocas' curve for the metabolic response to noradrenaline infusion during acclimation to cold and ours for the noradrenaline excretion in function of the time of exposure to cold is obvious The time course of the two phenomena is quite similar These observations can be related to other changes associated with heat production and observed during acclimation to cold, as increased food consumption, increase in survival at lethal temperature, increase in resistance to body cooling and decrease in shivering (*cf* HART 1960) It seems therefore reasonable to conclude that cold acclimation leads to an increased sensitivity to the calorigenic effect of endogenous noradrenaline, as judged by the decline in the excretion of this amine during acclimation to cold

Rats exposed to cold show an abrupt raise in their oxygen consumption (HART HEROUX and DEPOCAS 1956) which actually increases by a small percentage during the first 3—5 weeks (SELLERS and YOU 1950 COTTLE and CARLSON 1954) and remains high as long as rats are kept in the cold (up to 18 months) (SELLERS 1957) From the results of COTTLE and CARLSON (1954), it

especially parallel experiments, to correlate the decline in metabolism with the decline in noradrenaline excretion

The time course for the decrease in the noradrenaline excretion is in fact slightly longer than for the development of maximal metabolic response to

catecholamines, do not excrete more free noradrenaline and adrenaline than normal rats after injection of these substances These results also show that the inactivation processes are not activated by cold acclimation, which could have been an explanation for the decline in the catecholamine excretion on prolonged exposure to cold They also rule out the possibility that injected noradrenaline is being appreciably methylated in the animal and that this is what has happened in terms of its colorigenic action demonstrated in cold-acclimated rats after parenteral administration (HSIEH and CARLSON 1957, DEPOCAS 1960 a, b)

The above mentioned results on the inactivation of catecholamines in rats exposed to cold, and the observation that diuresis does not significantly affect the renal excretion of catecholamines (*cf* also PITKANEN 1956) indicate that the excretion of catecholamines in cold-exposed rats bears a close relationship to the actual secretion of these substances

The rate of adrenaline resynthesis is accelerated in rats exposed to cold but not the first day, which can explain the initial depletion in the glands The high resynthesis rate during the first week of exposure to + 3° C associated with a fairly normal suprarenal content gives further illustration of the difficulty in judging of the resynthesis from the actual content of the glands and emphasizes the importance of measuring simultaneously the output in urine As suggested by UDENFRIEND and WYNGAARDEN (1956), the synthesis can take place at a rapid rate but most of the newly synthetized material can be continually secreted into the blood stream during stimulation, only small amounts being taken up for storage in the glands This has been shown to be the fact by BYGDEMAN, EULER and HOKFELT (1960)

The variations in the adrenaline resynthesis with the temperature of exposure to cold and the body weight of animals pertain to the supplementary role of this hormone in the defense against cold It is evident that the normal values found for the noradrenaline content of the glands do not exclude a suprarenal secretion of this amine in response to cold stress It appears from some experiments at — 7° C that under severe conditions the noradrenal[illegible] stores of the [illegible]

Inc[illegible] [illegible]ipation of non visceral tissues as a possible site for chemical thermogenesis was given by DEPOCAS (1958, 1960 a, b) In cold-acclimated rats functional evisceration did not prevent the immediate raise in oxygen consumption seen on exposure to cold and the increase in oxygen consumption obtained on infusion of noradrenaline These observations poine to skeletal muscle, comprising about 40 % of the body mass, as the other tissud in which non shivering thermogenesis could take place The recently observen increased ability of muscle tissue of cold acclimated rats to take up oxyged *in vitro* (DAVIS, JOHNSTON, BELL and CREMER 1960) adds further support to this view The normal noradrenaline content of skeletal muscle in cold acclimated

adrenaline The organism can temporarily secrete more adrenaline than what is resynthetized by depleting the adrenal glands, as seen the first day of exposure to cold For noradrenaline the secretion is more dependent on the actual resynthesis

In the light of these considerations, the adrenaline excretion in rats exposed to cold can be better understood For instance, in rats of 170—180 g exposed to + 3° C the noradrenaline excretion is immediately nearly maximal so that, if more catecholamines are needed thereafter, most of the additional demand should be mediated through the release of adrenaline still available It is very likely that the duration as well as the intensity of cold stress increases the catecholamine requirements Indeed, from our experiments, it appears that the first week of exposure to cold is critical During that period rats are in a poor general condition and almost all deaths occur during that time This will explain the increased excretion of adrenaline during the first week of exposure With the development of an increased sensitivity to noradrenaline with the time in the cold (DEPOCAS 1960 a, b), less adrenaline is required to satisfy the catecholamine needs and therefore its secretion diminishes The decrease in the adrenaline output can also be partly due to the increased sensitivity of cold-acclimated rats to this substance (RING 1942, HSIEH and CARLSON 1957, SWANSON 1957) If the conditions are changed by varying either the temperature of exposure or the weight of animals, the maximal synthesis and/or secretion of noradrenaline is reached more of less rapidly according to these factors, and consequently the adrenaline secretion is more or less important according to the severity of the conditions For example, young rats exposed to — 7° C for 24 hours excrete more adrenaline than old ones after 6 days at + 3° C

As to the role of dopamine in the response to cold stress, it is still difficult to draw conclusions whether it has an independent role or it is mostly a precursor of catecholamines The increased excretion of this amine in the same conditions in which the synthesis and/or secretion of noradrenaline is maximal is not incompatible with the assumption that dopamine is a precursor of this hormone, but it can also be regarded as a compensatory mechanism In view of the large amounts excreted in urine, it would be surprising that it is merely a precursor of other catecholamines

It has been suggested (BROWN and GILLESPIE 1957) that the binding of the adrenergic transmitter with the receptors is an important step in the inactivation at/or near the site of release Moreover, the receptors would have a limited capacity to take up the transmitter, so that under conditions of strong stimulation the proportion of the transmitter escaping into the blood would be greater and hence the inactivation less complete, indicating a threshold effect It could therefore mean that the higher excretion rate of noradrenaline in rats exposed to cold indicates a smaller than proportional release (*cf* EULER 1959) This view is not supported by the recovery experiments after injection of catecholamines Cold exposed rats, which already excrete large amounts of

CHAPTER IV

EFFECT OF ACCLIMATION TO COLD ON THE PRODUCTION AND RELEASE OF CATECHOLAMINES

It is generally understood that animals show acclimation to cold in the sense that they undergo measurable physiological changes that make them more successful in withstanding cold stress Acclimation to cold is now well demonstrated in rats and its development is a gradual process requiring a period of time usually estimated to be from 2 to 6 weeks by various observers (*cf* HART 1960) During this time there is a gradual increase in cold resistance (BLAIR, DIMITROFF and HINGELEY 1951, HART 1953), an increase in food consumption (SEALANDER 1952, COTTLE and CARLSON 1954), an elevation of basal metabolic rate (many authors), an elevation of peripheral temperature (CARLSON 1954, HÉROUX 1959), a decrease in shivering and an increase in non shivering heat production (SELLERS, SCOTT and THOMAS 1954, COTTLE and CARLSON 1956, HART, HÉROUX and DEPOCAS 1956, HÉROUX, HART and DEPOCAS 1956, DAVIS, JOHNSTON, BELL and CREMER 1960) and other associated physiological and biochemical changes Therefore, acclimation to cold can be divided in two phases the process of acclimation, that means the gradual physiological modifications which lead to the obtention of a new equilibrium, the state of acclimation This stabilization at a new level is generally for a limited period of time It persists after removal from the cold for some weeks in some degree, although it diminishes rather quickly (SELLERS, REICHMAN and THOMAS 1951)

We have seen in the preceding chapter that prolonged exposure to cold led to a steady state in the excretion of catecholamines which persisted as long as rats were kept in the cold and was presumably due to an increased sensitivity of cold acclimated rats to catecholamines These modifications can possibly be regarded as adaptive changes, and we shall now consider how they alter the [illegible]

transferred to room temperature for some days and then re-exposed to the same temperature In the second series, the effect of previous exposure to + 3° C on the urinary excretion of catecholamines and the content of organs when animals were subjected to a temperature of — 7° C were studied

Methods

Re-exposure to the same temperature Male rats weighing on an average 180 g were exposed to cold at + 3° C as usual After six days in the cold, 18 rats were withdrawn and maintained at room temperature (+ 22° C) for 1 day (6 rats), 4 days (6 rats) or

rats does not exclude an accelerated rate of synthesis The large amounts of dopamine found in this tissue on exposure to cold can possibly be regarded as a sign of accelerated resynthesis of catecholamines *The time course of this* phenomenon parallels the development of the increased metabolic response to noradrenaline infusion (DEPOCAS 1960 a, b), but we do not have any direct evidence yet that the skeletal muscle of cold-acclimated rats shows an increased sensitivity to catecholamines Nevertheless, these observations suggest a rapid turnover of catecholamines in skeletal muscle of cold exposed rats, which may possibly be related to heat production The same changes also occur in the liver but to a lesser extent Moreover, since this organ represents only 3 % of the body mass, its contribution to the elevation of the oxygen consumption rate at the organismal level may be difficult to estimate in eviscerated animals

HÉROUX, DEPOCAS and HART (1959) have reported an increased cold resistance *in winter white rats kept outdoors This winter acclimatization was associated* with a greater capacity to produce heat and therefore resembles laboratory *acclimation to cold in this respect* Moreover, GELINEO and KOCAROV *(1955)* have shown seasonal changes in metabolism at constant acclimation temperature The quantitative seasonal changes in the catecholamine excretion on exposure to cold may well play a role there

Summary

The catecholamine production and release in response to acute and chronic cold stress was studied by measuring the output in urine in conjunction with the content of organs

Exposure to cold elicited an immediate, large increase in the noradrenaline excretion which persisted as long as rats were kept in the cold, although it slowly declined with time The noradrenaline excretion bore a close relationship to the time of exposure to cold, which was interpreted as a sign of increased sensitivity of cold-acclimated rats to this hormone The adrenaline excretion gradually rose to a maximum reached in about one week in the cold and decreased rapidly thereafter This was associated with a high rate of resynthesis in the adrenal glands These results are discussed in relation to the regulation *of non-shivering heat production in exposure and* acclimation to cold

The catecholamine excretion in cold-exposed rats varied with the severity of cold stimuli Under the present experimental conditions, the adrenaline output was found to be linearly related either to the temperature of exposure or the body weight of animals On the contrary, the noradrenaline excretion rapidly arrived at a plateau level when the conditions became more severe, suggesting a limited synthesis and/or secretion of this hormone The hypothesis is put forward that adrenaline acts as a second line of defense against cold which need not be called on for great activity unless the noradrenaline mechanism becomes saturated

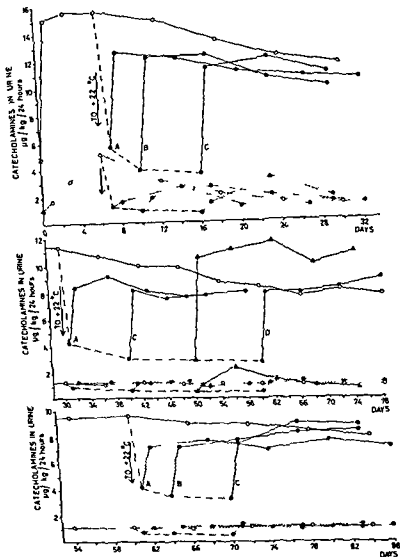


Fig 14 [illegible]

was slightly lower than that of the control group maintained in the cold (Fig 14) Thereafter the noradrenaline curves of the re-exposed groups paralleled the control ones In the group of rats which have spent one month at room tempera-

10 days (6 rats) before being re exposed to the same temperature Thirty animals acclimated to + 3° C for one month were transferred to + 22° C and re exposed to cold after 1, 4, 10 or 30 days (6 rats per group) Similar experiments were conducted with 2 month acclimated rats (18 rats) Six animals were continuously maintained in the cold and served as controls In a group of six rats acclimated to cold at + 3° C for one month and re exposed to the same temperature after 10 days at + 22° C the catecholamine content of organs the first day of re-exposure was studied In other cases only the urinary excretion of amines has been followed

Acute exposure to — 7° C Warm acclimated rats (+ 22° C) and cold acclimated ones (+ 3° C) of about the same weight were exposed to cold at — 7° C for 24 hours and compared as regard to the urinary excretion of catecholamines and the content of organs There were six groups (six rats per group) of intact warm and cold acclimated animals for different periods of time and two groups of warm and cold acclimated (6 weeks at + 3° C) adrenalectomized rats receiving corticoid therapy (2 mg of desoxy corticosterone and cortisone per rat per day) For the sake of comparison all groups of cold acclimated rats have spent 24 hours at room temperature before the test at — 7 C For the catecholamine content of organs comparisons were made on a relative basis For example, the organ content of 90 day cold acclimated rats exposed to — 7° C was first compared with that of an identical group transferred at room temperature The differences were then compared to those observed between warm acclimated animals before and after exposure to — 7° C

Chronic exposure to — 7° C Five groups of six animals were compared regarding the urinary excretion of catecholamines and the content of organs on chronic exposure to — 7° C The test period was limited to 12 days The first group consisted of warm acclimated (+ 22° C) rats of an average weight of 305 g the second one of rats which have been in the cold at + 3° C for one week (average weight 275 g) the third one of rats exposed to + 3° C for 2 weeks (average weight 293 g) the fourth one of cold acclimated rats for 4 weeks at + 3° C (average weight 326 g) and the fifth one of 12 week 3° C acclimated rats weighing on the average 376 g All groups of rats which have been previously exposed to + 3° C have spent 24 hours at room temperature before the test so that the excretion values of catecholamines were at about the normal levels at the beginning of experiments If an animal died during the collection of urine the values for this rat were corrected for a 24-hour period In those cases where rats died in the cold organs were removed as soon as possible after death and frozen till the time of extraction In all these experiments at — 7° C glycerol (15 % v/v) was added to the drinking water to prevent the formation of ice

Results

Catecholamine excretion and content of organs on re exposure to the temperature of acclimation

When rats were removed from the cold room at + 3 C the catecholamine excretion values returned to normal within two days at room temperature The slightly higher values found during the first 24 hours at + 22 C may be due to incomplete emptying of the bladder and/or to catecholamines still present in body fluids

Rats which have been in the cold room at + 3 C for one week one month or two months on re exposure to the same temperature after one four or ten days at room temperature increased their noradrenaline excretion to a level which

Table IX Urinary excretion of catecholamines in different groups of warm- and cold-acclimated rats on acute exposure to − 7° C All groups of cold-acclimated rats have spent one day at room temperature before the test Six rats per group

Groups	24 hours at + 22° C			24 hours at − 7° C		
	Nor	Adr	Dop	Nor	Adr	Dop
	µg/kg/24 h Mean ± S E.			µg/kg/24 h Mean ± S E.		
Warm acclimated + 22° C, 36 days, 303 g	3.0 ± 0.11	0.62 ± 0.03	41 ± 3.4	13.6 ± 1.33	1.52 ± 0.13	46 ± 3.2
Cold acclimated + 3° C, 36 days, 280 g	3.3 ± 0.11	0.78 ± 0.07	43 ± 3.8	11.5 ± 0.71	1.35 ± 0.09	41 ± 4.6
Warm acclimated + 22° C, 90 days, 356 g	2.8 ± 0.09	0.63 ± 0.04	41 ± 3.4	13.4 ± 0.98	1.98 ± 0.13	39 ± 3.2
Cold acclimated + 3° C, 90 days, 340 g	3.7 ± 0.14	0.72 ± 0.06	39 ± 3.0	10.9 ± 1.08	1.34 ± 0.07	42 ± 2.8
Warm acclimated + 22° C, 36 days, 175 g	4.0 ± 0.07	0.70 ± 0.03	43 ± 2.1	15.0 ± 0.91	3.24 ± 0.25	47 ± 4.2
Cold acclimated + 3° C, 30 days, 190 g	4.0 ± 0.05	0.78 ± 0.03	39 ± 2.5	11.9 ± 1.04	2.02 ± 0.10	41 ± 2.8

Table X Catecholamine content of adrenal glands and adrenaline secretion and resynthesis in different groups of warm- and cold-acclimated rats exposed to − 7° C for 24 hours Six rats per group

Groups	Adrenal glands		Adrenaline	
	Nor-adrenaline	Adrenaline	Secretion	Resynthesis
	µg/kg b. wt. Mean ± S E.		µg/kg/24 h	
Warm acclimated + 22° C, 36 days, 303 g	16.2 ± 1.3	134 ± 2.8	49	55
Cold acclimated + 3° C, 36 days, 280 g	16.2 ± 1.3	127 ± 3.5	44	43
Warm acclimated + 22° C, 90 days, 356 g	14.2 ± 1.0	136 ± 4.2	63	72
Cold-acclimated + 3° C, 90 days, 340 g	18.3 ± 1.7	127 ± 4.9	43	43
Warm acclimated + 22° C, 36 days, 175 g	18.2 ± 1.6	97 ± 4.3	104	79
Cold acclimated + 3° C, 30 days, 190 g	18.4 ± 1.2	126 ± 4.0	65	67

cold-acclimated rats except in the spleen, where there was an increased concentration of adrenaline in cold-acclimated animals (Table XI). The adrenaline depletion in heart, spleen and liver of adrenalectomized rats was more pronounced than in intact animals

ture before re-exposure to cold the noradrenaline excretion rose to the same level as the control one

The adrenaline excretion increased to the control level in all groups of rats acclimated to cold for one or two months In these two series there was no significant variations in the dopamine output on re-exposure to cold On the other hand, in all groups of animals previously exposed to cold for only one week the adrenaline excretion showed a definite peak the sixth day of re-exposure Moreover, *in the group re-exposed to cold* after 10 days at room temperature there was a significant increase in the dopamine excretion

Generally, on re-exposure to cold rats increased their excretion of adrenaline and noradrenaline to about the same levels as the control group maintained in the cold, but never as high as rats of the same weight exposed to cold for the first time of their life This was true for rats which have been in the cold for only one week, in which the noradrenaline excretion and the peak in the adrenaline excretion were lower than the first time of exposure, as well as for acclimated animals kept 30 days at room temperature before re-exposure to cold, in which the noradrenaline and adrenaline excretion values were as high as in the control group in the cold, but significantly lower than the values observed in rats of about the same weight exposed to cold for the first time (*cf* Fig 14)

The variations in organs were also in the same direction In a group of rats acclimated to + 3° C for one month and then re-exposed to cold after 10 days at room temperature, there was no depletion in the adrenaline content of adrenal glands the first day of re exposure and the noradrenaline depletion in other organs was much less than in rats exposed to cold for the first time

Catecholamine excretion and content of organs on acute exposure to — 7° C

In all cases, cold-acclimated animals excreted less noradrenaline and adrenaline than warm-acclimated rats of about the same weight on *acute exposure* to — 7° C (Table IX) However, the differences, although significant, were not very large The dopamine output did not show any significant difference in these experiments

In the adrenal glands of young warm-acclimated rats exposed to — 7° C there was a depletion in the adrenaline content while no change occurred in the cold-acclimated group of about the same weight There was therefore a significant *difference between these two* groups (Table X) However, the noradrenaline concentrations were identical In other groups the values for both adrenaline and noradrenaline were very close The rate of resynthesis of adrenaline, calculated from the excretion values in urine and the actual content of the glands after 24 hours in the cold, was slightly higher in the warm-acclimated groups than in the cold-acclimated ones

In other organs, comparisons between the relative changes in the concentrations of adrenaline and noradrenaline showed no differences between warm- and

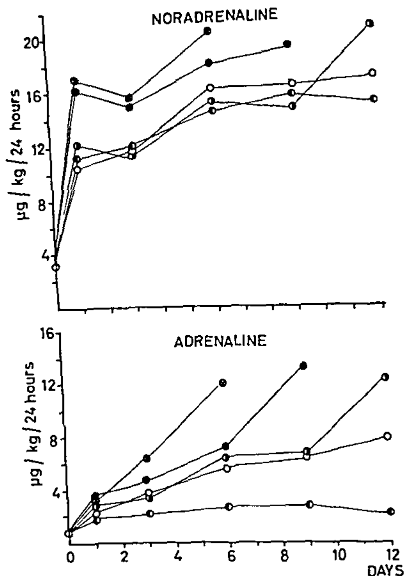


Fig 15 Excretion of adrenaline and noradrenaline on prolonged exposure to — 7° C in rats which have never been in the cold before (⊗) or at + 3° C for 1 (●) 2 (◑), 4 (○) and 12 (◐) weeks There were six rats per group at the beginning of experiments

week acclimated group, but they were all hypothermic at that time (mean colonic temperature 33 2° C) Rats previously acclimated for 12 weeks at + 3 C were all alive and normothermic at the end of the 12 day test at — 7° C, although they all have lost weight and some of them showed local tissue injuries

Table XI Catecholamine variations in heart, spleen and liver of different groups of warm and cold-acclimated rats exposed to — 7° C for 24 hours Results expressed in per cent variation between the experimental group and a control group at room temperature Six rats per group

Groups	Heart		Spleen			Liver		
	Nor	Adr	Nor	Adr	Dop	Nor	Adr	Dop
Warm acclimated + 22° C, 36 days, 175 g	−59	−29	−16	− 5	−100	−18	−10	+606
Cold acclimated + 3° C, 30 days, 190 g	−56	−34	−23	+18	*	−14	−19	+907
Warm-acclimated + 22° C, 90 days, 356 g	−42	−17	−12	− 7	−100	−22	−21	+516
Cold acclimated + 3° C, 90 days, 340 g	−33	−29	−15	+20	*	−23	−20	+583
Adrenalectomized Warm acclimated + 22° C, 42 days, 338 g	−50	−62	−20	−16	− 60	−42	−75	+358
Adrenalectomized Cold acclimated + 3° C 42 days 292 g	−31	−71	−12	− 2	+660	−34	−89	+642

* There was no dopamine in the spleen at the time of exposure to — 7° C in these two groups of cold acclimated rats

Dopamine completely disappeared from the spleen of warm-acclimated rats during the first 24 hours at — 7° C while, as previously shown (*cf* Chapter III), there was still some dopamine in the spleen after 24 hours at + 3 C In cold-acclimated rats there was already no dopamine in the spleen before exposure to — 7° C except in the group of adrenalectomized animals In this latter group a large increase in the dopamine content of the spleen was observed, but a 60 % decrease in the warm-acclimated adrenalectomized group In all groups the dopamine content of the liver increased on exposure to — 7° C but more in the cold-acclimated groups than in the warm-acclimated ones

Catecholamine excretion and content of organs on prolonged exposure to — 7 C

The survival of rats chronically exposed to — 7° C was found to be related to their previous history of acclimation to + 3° C All rats which have never been in the cold before died within 8 days at — 7° C (average survival time 170 hours) Of those exposed to + 3° C for one week before, only two survived 9 days (average survival time 209 hours) If rats have spent two weeks at + 3 C, they could live on an average 241 hours One rat was alive the twelfth day but hypothermic Five rats were still living after 12 days at — 7° C in the 4-

rats dead in the cold both amines were largely depleted whereas the concentrations were higher than normal in cold-acclimated animals The resynthesis of adrenaline in warm acclimated rats at the time of death in the cold was between [illegible]

[illegible]

the adrenaline content showed a tendency to increase in warm-acclimated rats dead in the cold Both amines were in quite normal concentrations in organs of cold acclimated rats the sixth and the twelfth day at — 7° C

Discussion

The lower excretion of noradrenaline brought about by prolonged exposure to cold can definitely be regarded as an adaptive change Cold acclimated rats re-exposed to the same or a lower environment excrete less noradrenaline than warm acclimated animals under similar conditions Since considerable evidence has accumulated showing that the metabolic response in cold acclimated rats is not associated with physical activity of the skeletal muscle (SELLERS, SCOTT and THOMAS 1954, COTTLE and CARLSON 1956, HÉROUX, HART and DEPOCAS 1956, HART, HÉROUX and DEPOCAS 1956), these results also support the idea of noradrenaline as the mediator of non shivering thermogenesis (HSIEH and CARLSON 1957)

That acclimation to cold brings about an increased sensitivity to the metabolic effect of noradrenaline (HSIEH and CARLSON 1957, DEPOCAS 1960 a, *b*) is illustrated by the higher metabolic rate commonly observed in cold acclimated rats exposed to cold (SELLERS, REICHMAN, THOMAS and YOU 1951, COTTLE and CARLSON 1956, HEROUX, HART and DEPOCAS 1956) associated with a lower excretion of noradrenaline The main change in acclimation to cold may well be an increased sensitivity of the tissues to the calorigenic effect of noradrenaline, and consequently a decreased secretion of this *hormone* The adrenaline secretion may be diminished partly because of the reduced needs for its supplementary metabolic effects (*cf* Chapter III) and partly because of the increased sensitivity of cold-acclimated rats to this hormone (RING 1942, HSIEH and CARLSON 1957, SWANSON 1957)

[illegible]

ex [illegible]

be [illegible] degree of acclimation is, however, achieved after two or even one week of exposure It also appears from our observations at — 7° C that acclimation to cold should be defined as specifically applying not to the peak change in the excretion of catecholamines which occurs after about one month in rats exposed at + 3° C (*cf* Chapter III), but to a later period when plateau values are well above normal but lower than the peak

The adaptive changes in the excretion of catecholamines persist for a long time after removal from the cold In rats kept at room temperature for one month

Table XII Catecholamine content of organs in warm (+ 22° C) and cold (+ 3° C) acclimated rats on chronic exposure to − 7° C Three animals at each time

Groups	Adrenals		Heart		Spleen		Liver		Muscle	
	Nor	Adr	Nor	Adr	Nor	Adr	Nor	Adr	Nor	Adr
	μg/kg b wt		μg/g tissue		μg/g tissue		μg/g tissue		μg/g tissue	
Warm acclimated										
+ 22° C control	22 8	150	0 55	0 032	0 67	0 027	0 044	0 002	0 054	0 003
− 7° C 1st day	17 1	138	0 21	0 015	0 32	0 024	0 030	0 002	0 028	0 002
− 7° C 5th day[1]	15 9	78	0 28	0 018	0 40	0 064	0 017	0 006	0 036	0 004
− 7° C 7th day[1]	14 2	56	0 25	0 030	0 39	0 054	0 035	0 004	0 040	0 003
Cold acclimated										
+ 22° C control	19 9	173	0 58	0 038	0 65	0 067	0 050	0 006	0 057	0 003
− 7° C 1st day	18 3	167	0 23	0 014	0 43	0 071	0 035	0 004	0 038	0 003
− 7° C 6th day	21 0	201	0 41	0 039	0 78	0 073	0 047	0 005	0 070	0 004
− 7° C 12th day	25 1	226	0 41	0 038	0 51	0 044	0 042	0 007	0 053	0 003

[1] Catecholamines estimated after the death of animals in the cold

Fig 15 shows the excretion of adrenaline and noradrenaline for these five groups of rats exposed to − 7° C Time 0 corresponds to the day at 22° C, and since the excretion values for the different groups were very close only those for the 4 week acclimated group were represented at that time In rats which have never been in the cold before or only one week at + 3° C the noradrenaline excretion immediately attained maximal values In other groups the excretion was significantly lower for the first three days, but thereafter it increased to the maximal level On the other hand adrenaline showed a gradual increase toward a plateau, around 12 μg/kg/24 hours, except in the group previously acclimated for three months at + 3° C This maximal excretion of adrenaline was reached more or less rapidly according to the previous history of acclimation to + 3° C and was closely associated with the death of animals It was not, however, necessarily related with hypothermia In many cases rats with a fairly normal colonic temperature excreted maximal amounts of adrenaline Generally, hypothermia appeared soon after the limit for the adrenaline excretion has been reached In all groups the dopamine output gradually increased with the time of exposure, but not to a constant level The dopamine excretion was inversely related to the previous acclimation time at + 3° C

Table XII shows the catecholamine concentrations in organs of warm acclimated rats and 12-week cold acclimated ones at different times after exposure to − 7° C As previously mentioned small differences were seen between the two groups the first day of exposure to − 7° C However, marked differences appeared on continuation of exposure In the adrenal glands of non acclimated

In the preceding chapter we have mentioned the supplementary role of adrenaline and presumed that its secretion was also limited This limit has been reached in chronic experiments at — 7° C It is illustrated by the large depletion in the adrenaline content of adrenal glands associated with a fairly constant urinary output around 12 µg/kg/24 hours, corresponding to a secretion of about 375—400 µg/kg/24 hours and a resynthesis of 280—300 µg/kg/24 hours The observation that maximal adrenaline secretion generally occurs before hypothermia develops suggests that hypothermia and death result from the saturation of the mechanisms for the synthesis and/or secretion of adrenaline and noradrenaline

Since all rats, warm or cold acclimated, increase their catecholamine excretion to the same maximal level, it means that cold acclimation does not enhance the maximal capability for the synthesis and/or secretion of these substances Therefore, the increased ability of cold acclimated rats to maintain high rates of heat production in colder environments (SELLERS REICHMAN, THOMAS and YOU 1951) and the greater maximal thermogenic capacity of these animals (DEPOCAS HART and HÉROUX 1957) are really due to a greater sensitivity to catecholamines The striking effect of acclimation to cold may be a sparing action on the secretion and/or synthesis of adrenaline and noradrenaline by virtue of an increased sensitivity of cold acclimated animals to catecholamines Cold acclimation would then extend the time and the temperature at which both mechanisms would be saturated, and consequently would prolong survival in a colder environment

Summary

Cold acclimated rats re-exposed to the temperature of acclimation or to a lower environment excreted less catecholamines than warm acclimated animals The catecholamine response of rats chronically subjected to a temperature of — 7° C was found to be closely related to the previous history of acclimation to + 3° C These findings are interpreted as showing that the main change brought about by cold acclimation may well be an increased sensitivity of the tissues to the calorigenic effects of catecholamines

Acclimation to cold was, however, limited This could be gathered from the failure of + 3° C acclimated rats to maintain low excretion levels of catecholamines on prolonged exposure to — 7° C and from the mortality, loss in weight and poor general condition of these rats The limitation of cold acclimation seems to result from a saturation of the mechanisms for the synthesis and/or secretion of noradrenaline and adrenaline.

Further evidence is given for the supplementary role of adrenaline It appears that cold acclimation by virtue of an increased sensitivity to catecholamines especially noradrenaline, exerts a sparing action on the synthesis and/or secretion of adrenaline and noradrenaline, thus extending the time and the temperature at which both mechanisms are exhausted

after acclimation the noradrenaline excretion on re-exposure to the same temperature is well below the level of non-acclimated rats, although it is higher than in other groups of acclimated rats re-exposed after a few days at room temperature As judged by survival in the cold (1 5 ° C) after removal of the fur by clipping, SELLERS, REICHMAN and THOMAS (1951) found little acclimation in rats maintained for 1 to 14 days in the cold, but considerable acclimation after 4 to 6 weeks Such acclimation persisted after removal from the cold for some weeks in some degree, although it diminished rather quickly Much of the increased resistance has disappeared in animals maintained four days in a warm environment (30° C) In our case the test consisted in re-exposing animals under the same conditions, and the persistence of a low degree of acclimation may explain the results It will be seen later (*cf* Chapter VII) that reduction of insulation by removal of the fur greatly affects the acclimation process

The chronic experiments at — 7° C, besides emphasizing the importance of the duration of exposure to cold as well as the environmental temperature on the excretion of catecholamines, point to an aspect of cold acclimation which is sometimes misunderstood the limitations of acclimation to cold HART, HEROUX and DEPOCAS (1956) have shown that the capability for a physical response in cold-acclimated rats, as indicated by the electromyogram, was not abolished since shivering showed up when they were moved to a lower temperature Moreover, acclimation to + 6° C did not allow acclimation to — 6° C, as shown by the failure of shivering to disappear in rats kept for 5 weeks at — 6° C Using the catecholamine excretion as a criterion of cold acclimation, similar conclusions can be drawn from our results The view has been propounded that acclimation to cold leads to an increased sensitivity to the noradrenaline effects with consequently a lower secretion of this amine However, cold-acclimated rats can still increase their noradrenaline secretion to maximal values as well as normal animals when the conditions become more severe Moreover, the increased sensitivity to noradrenaline can help but does not necessarily allow acclimation to a new environment, as seen in the two groups of cold-acclimated rats (4 and 12 weeks) exposed to — 7° C

The observation of DEPOCAS, HART and HEROUX (1957) that acclimation to cold extends the range of temperatures at which the initial response in heat production is adequate for temporary maintenance of thermal balance, but with eventual breakdown in the heat production mechanisms and resultant progressive hypothermia and death can be interpreted in terms of catecholamines The breakdown in heat production seems to occur when the mechanisms for the noradrenaline and adrenaline synthesis and/or secretion are saturated The noradrenaline limit is quickly attained, as shown previously (*cf* Chapter III) and confirmed here in chronic experiments at — 7° C The maximal excretion for this amine is around 20 μg/kg/24 hours which corresponds to a secretion of about 600 μg/kg/24 hours If then additional catecholamines are needed, it should come from the adrenaline secretion which is still available

4 days and then re-exposed to cold Their catecholamine excretion was compared with that of a group of intact cold acclimated rats (8 weeks at + 3° C, 330 g) transferred to + 22° C and re exposed to + 3° C four days later

lasting effect Moreover it does not possess demonstrable adrenergic blocking or atropine-like properties and in comparison with d tubocurarine its curare like action is insignificant (STONE TORCHIANA NAVARRO and BEYER 1956)

Three series of experiments were conducted one on warm acclimated rats main- + 3° C climated The first

group received 40 mg/kg of mecamylamine subcutaneously The second group was first treated with L-noradrenaline hydrochloride (0 2 mg/kg subcutaneously) and 15 minutes later with the same dose of mecamylamine The third group was pre-treated with L-adrenaline hydrochloride (0 2 mg/kg subcutaneously) These animals were maintained at room temperature while 18 rats similarly treated were exposed to cold immediately

was followed every 15 or 30 minutes after mecamylamine treatment until the temperature had returned to normal

The effects of ganglionic blockade on the excretion of catecholamines and the content of organs were studied in three similar series of experiments on four groups of six rats The first group was the control group without any treatment The second third and

It should be mentioned that the ganglion blocking action of mecamylamine, at a dose of 20 mg/kg did not last for three hours as judged by the colonic temperature This schedule of 20 mg/kg every 3 hours was chosen to allow rats to live in the cold sufficiently long for an adequate collection of urine, otherwise catecholamines in urine could not be estimated accurately

Adrenergic blockad

with 10 mg/kg

French) intrape

groups of three ... 100 hours for the estimation of catecholamines in organs Urine was collected from six randomly assigned animals at different times after the beginning of treatment

th ... the cold acclimated rats (2 months at + 3° C, 345 g) and six adrenalectomized cold acclimated animals (6 weeks at + 3° C, 240 g) were similarly treated Three cold acclimated rats were also treated with a higher dose of phenoxybenzamine, 20 mg/kg day Catecholamines in urine and organs were measured at

CHAPTER V

ORIGIN AND IMPORTANCE OF CATECHOLAMINES IN THE RESPONSE TO COLD EXPOSURE

The increased output of catecholamines in rats exposed to cold is due to an increased secretion either from the adrenal glands or from other tissues or from both sources. Evidence has been presented that in man and various animals urinary adrenaline originates chiefly, but not exclusively, from the adrenal medulla while the adrenergic nerves are the main source of noradrenaline in urine (*cf.* EULER 1956, 1959). Similarly, it can be presumed that the increased adrenaline excretion in rats exposed to cold is mainly due to an increased secretion from the adrenal medulla while the increased noradrenaline output mostly results from an increased release from the adrenergic nerve endings.

Experiments were conducted in order to verify this assumption. Firstly, the contribution of the adrenal glands to the increased secretion of catecholamines on exposure to cold was estimated in adrenalectomized rats. Secondly, the release of catecholamines from adrenergic nerve endings was indirectly blocked by means of a ganglion-blocking agent. By this approach information on the importance of catecholamines in the response of rats to cold exposure was also obtained. This problem was further investigated by inhibiting the physiological effects of catecholamines at the receptor sites by using an adrenergic blocking agent.

Methods

Adrenalectomy. Bilateral adrenalectomy was performed in one stage by the dorsal approach under Nembutal anesthesia (35 mg/kg intraperitoneally). Three different treatments were instituted after operation. 12 old rats (average weight 400 g) received saline (1 % NaCl) *in lieu* of water while 30 young animals (average weight 185 g) were daily injected with cortical hormones: desoxycorticosterone acetate subcutaneously (Percorten, Ciba) and cortisone acetate intramuscularly (Cortone, Merck, Sharp and Dohme), 0.5 mg of each hormone per rat per day (18 animals) or 2.0 mg per rat per day (12 rats). Adrenalectomized rats were exposed to cold at + 3° C one week after operation except six of them receiving 0.5 mg/rat day of corticoids which were maintained at room temperature as controls. Twelve young rats (180 g) were sham operated and six were exposed to cold one week after operation while six were kept at room temperature.

The effect of adrenalectomy on the acclimation process was studied in a group of six cold acclimated rats (8 weeks at + 3° C, 340 g) transferred to room temperature, adrenalectomized and re-exposed to cold four days later. The catecholamine excretion of these rats was compared with that of cold acclimated animals re-exposed to cold ... In order to see if adrenalectomized rats could achieve ... intact animals, a group of six adrenalectomized cold ... 3° C, 310 g) were transferred to room temperature for

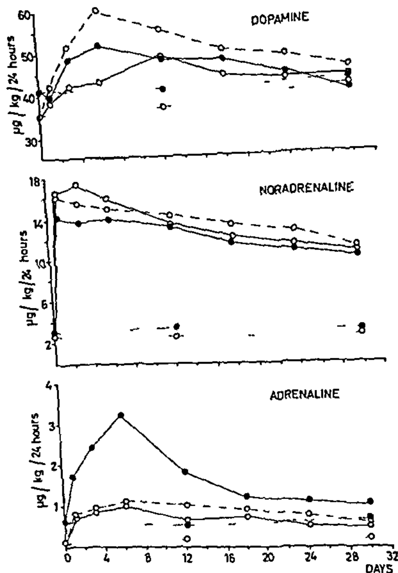


Fig 16 Catecholamine excretion in adrenalectomized (○) and sham-operated rats (●) at + 3 C (—) and + 22 C ( ) Cortical hormones given at doses of 0 5 mg (—) or ? 0 mg ( ) per rat per day Each point represents the mean of six rats

be detected in the spleen of cold acclimated rats while appreciable quantities were found in liver (3 ng g of tissue) and skeletal muscle (7 ng/g of tissue) as in intact animals after one month in the cold (*cf* Chapter III)

various times after exposure to cold The catecholamine content of organs was estimated after the death of animals in the cold Organs were immediately removed and frozen till the time of extraction In those cases where rats died during the collection of urine, the values were corrected for a 24-hour period

Results

Effect of adrenalectomy on the catecholamine response to cold exposure

The mortality of adrenalectomized rats exposed to cold was found to be mostly related to the corticoid therapy Eight animals maintained on saline and six receiving low dosages of cortical hormones died while ten of those treated with 2 mg of corticoids were still alive after one month at + 3° C Almost all deaths occurred during the first week of exposure to cold The colonic temperature of rats which died in the cold was subnormal for about 24 hours before the fatal issue, but in rats which survived the colonic temperature was at all times normal

The effect of adrenalectomy on the excretion of catecholamines in rats exposed to cold is shown in Fig 16 There was still a large increase in the noradrenaline output which was in fact greater than in the sham-operated group in the cold The curves also showed the same decline with the time of exposure as in the control group In contrast, the increase in the adrenaline excretion was about 80 % less in the adrenalectomized groups than in the sham-operated one However, the adrenaline output in adrenalectomized rats exposed to cold was significantly increased above the value found in the adrenalectomized group at room temperature The dopamine excretion was generally lower in adrenalectomized animals than in intact animals, but on exposure to cold adrenalectomized rats increased their dopamine excretion as much as sham-operated animals

Adrenalectomized cold-acclimated rats re-exposed to cold after 4 days at room temperature increased their catecholamine excretion to the same level as the control group re-exposed to cold The catecholamine excretion values were 7 3 μg/kg/24 hours for noradrenaline, 0 51 for adrenaline and 32 for dopamine the first day and 9 47, 1 04 and 29 μg/kg/24 hours the sixth day compared to 8 8, 0 59 and 27 μg/kg/24 hours for the control group When adrenalectomy was performed after acclimation to cold, the catecholamine excretion was higher than in the control group on re-exposure to cold, 10 7 μg/kg/24 hours for noradrenaline, 0 66 for adrenaline and 30 for dopamine the first day and 11 2, 1 24 and 33 μg/kg/24 hours the sixth day These values, however, were much lower than those observed in warm-acclimated adrenalectomized rats of about the same weight exposed to cold for the first time

In organs of adrenalectomized animals no differences in the noradrenaline and adrenaline concentrations, measured after 42 days, were found between warm- and cold acclimated rats *Only negligeable amounts of dopamine could*

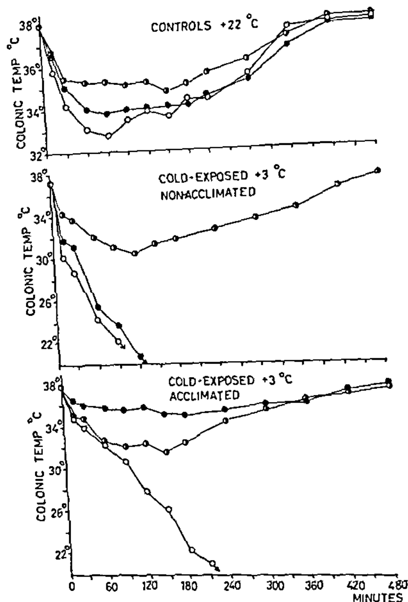


Fig 17 Effect of ganglionic blockade on the colonic temperature of intact rats (○), rats pre-treated with noradrenaline (●) and rats pre-treated with adrenaline (◑) Catecholamines (0 2 mg kg s. c) injected 15 minutes before mecamylamine (40 mg kg s c. in non acclimated rats and 80 mg kg s c in acclimated ones) Six rats per group

In order to further elucidate the origin of the extra-adrenal adrenaline, the effect of histamine (2 mg/kg subcutaneously) on the excretion of adrenaline in adrenalectomized rats was studied The histamine test was performed at 2, 4 and 8 weeks after operation in warm- and cold-acclimated rats In five instances, there was a slight, but not significant, increase (15—25 %) in the adrenaline excretion after histamine It should be pointed out that urine was collected for 24 hours after a single injection of histamine, and this can account for the low increase in the adrenaline values

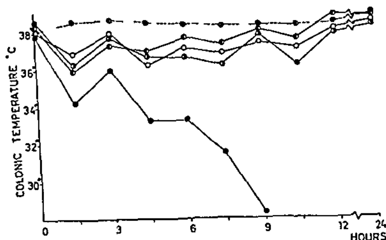
Effect of ganglionic blockade on the catecholamine response to cold exposure

The survival tests after injection of mecamylamine gave the following results The colonic temperature of rats during these experiments is shown in Fig 17. All warm-acclimated rats at room temperature survived after a single injection of 40 mg/kg of the ganglion-blocking agent Immediately after injection the colonic temperature dropped and reached a minimum between 60 and 90 minutes with a return to normal in about 7 hours Pre-treatment with noradrenaline did not significantly reduce the decrease in the colonic temperature, but adrenaline had a definite protective effect All these rats showed severe shivering after injection of mecamylamine, especially during the following 2 or 3 hours Shivering was less intense in rats pre-treated with adrenaline

Warm-acclimated mecamylamine treated rats exposed to cold died in hypothermia very quickly (average survival time 125 minutes) The colonic temperature decreased very rapidly and these rats shivered tremendously Pre-treatment with noradrenaline did not significantly prolong the survival time (137 minutes) Shivering was also very severe and the colonic temperature decreased rapidly In the group receiving adrenaline before mecamylamine, only two rats died in the cold between the third and the fourth hour The decrease in the colonic temperature and shivering were less marked than in the two other groups

Cold-acclimated rats treated with 80 mg/kg of mecamylamine all died in hypothermia in an average of 260 minutes Shivering was very intense and equivalent to that found in warm-acclimated rats Adrenaline still had a protective effect, only one rat died after 3 hours The colonic temperature in this group did not decrease more than in warm-acclimated rats, although the mecamylamine dosage was twice as high Shivering was still visible but less severe All cold-acclimated animals pre-treated with noradrenaline survived after mecamylamine The colonic temperature showed a relatively small decrease and shivering was intermittent and not severe

In summary, adrenaline had a protective action on the hypothermia produced by mecamylamine in all groups of rats, but this effect was more pronounced in cold-acclimated rats Noradrenaline did not reduce the decrease in the colonic temperature caused by ganglionic blockade in warm-acclimated rats either at room temperature or in the cold In cold-acclimated rats, however,



mecamylamine treatment except in cold exposed rats injected with the highest dose, where there was a decrease in the dopamine output

The results on the excretion of catecholamines after mecamylamine treatment can be better understood by referring to the temperature curves (Fig 18) As judged by the colonic temperature, the action of mecamylamine at a dose of 20 mg/kg did not last for three hours The colonic temperature dropped immediately after injection and reached a minimum in about 1 1/2 hour Thereafter there was a gradual return to the normal level Therefore, at the dosages used in these experiments the blockade was neither complete nor continuous

Warm acclimated adrenalectomized rats exposed to cold died in an average of 13 hours during treatment with mecamylamine (20 mg/kg every 3 hours) The catecholamine excretion values for the first 12 hours were 1 3 μg/kg for noradrenaline, 0 23 μg/kg for adrenaline and 10 μg/kg for dopamine Cold-acclimated adrenalectomized animals were also more sensitive to cold after mecamylamine, three rats out of six died in hypothermia between the twelfth and the sixteenth hour The excretion values for noradrenaline, adrenaline and dopamine were 1 5, 0 20 and 11 μg/kg respectively during the first 12 hours and 1 9, 0 57 and 13 μg/kg during the second 12 hour period Needless to say that all these values were significantly lower than those observed in non-treated adrenalectomized animals on exposure to cold

The catecholamine concentrations in organs of rats treated with a ganglion-blocking agent are shown in Table XIV In rats at room temperature, killed 24 hours after the first injection of mecamylamine (20 mg/kg every 3 hours for

Table XIII Catecholamine excretion after ganglionic blockade in rats at room temperature (+22° C) and in the cold at + 3° C Mecamylamine was injected every 3 hours during the first 12-hour period Six rats per group

	Control (no treatment)			Mecamylamine (subcutaneous injection every 3 h for 12 h)								
				5 mg/kg			10 mg/kg			20 mg/kg		
	Nor	Adr	Dop	Nor	Adr	Dop	Nor	Adr	Dop	Nor	Adr	Dop
	µg/kg b wt			µg/kg b wt.			µg/kg b wt			µg/kg b wt		
Warm acclimated rats + 22° C												
1st 12 h	2 2	0 28	18	1 9	0 40	18	1 2	0 35	15	1 3	0 52	18
2nd 12 h	2 6	0 31	20	2 3	0 23	25	2 1	0 32	19	2 0	0 78	23
Warm acclimated rats + 3° C												
1st 12 h	8 7	0 88	16	4 3	2 00	19	3 0	2 65	20	1 2	0 89	12
2nd 12 h	8 9	1 10	19	8 8	3 60	24	7 5	5 12	20			
Cold acclimated rats + 3° C												
1st 12 h	4 3	0 40	16	3.5	0 92	14	2 6	1 15	18	2 0	0 80	10
2nd 12 h	4 9	0 50	17	7 1	1 92	16	6 4	2 32	19	5 8	3 38	16

noradrenaline exerted a marked protection, as judged by the survival of these rats and the slight decrease in the colonic temperature

The excretion of catecholamines after ganglionic blockade is shown in Table XIII At the three dosages used, mecamylamine reduced the noradrenaline output during the period of treatment in the three series of animals The decrease was proportionate to the dosage, suggesting an incomplete blockade with the doses used The effect of mecamylamine on the excretion of noradrenaline was more marked in warm-acclimated rats exposed to cold *All animals treated with 20 mg/kg every 3 hours died in the cold (average survival time 10 hours)* During the second 12-hour period the noradrenaline excretion rose to values which were slightly lower than in the control groups in warm-acclimated rats at room temperature or in the cold but higher in the cold-acclimated groups The noradrenaline excretion values during that period were inversely proportionate to the mecamylamine dosage

The excretion of adrenaline increased even during treatment with mecamylamine, especially in cold-exposed animals, but this increase was not related to the dosage In the recovery period, there was a large increase in the adrenaline output of rats in the cold, particularly non-acclimated ones The adrenaline excretion was there proportionate to the dosage, and it is interesting to note the inverse relationship between the noradrenaline and adrenaline excretions during the second 12 hours The dopamine excretion was not greatly affected by

Table XVI Urinary excretion of catecholamines after adrenergic blockade produced by phenoxybenzamine in warm and cold acclimated rats in the cold at + 3° C compared to the control group maintained at room temperature The adrenolytic agent was used at a dose of 10 mg/kg/day i p Day 0 indicates day before treatment

Days	No of rats	Nor	Adr	Dop	No of rats	Nor	Adr	Dop	No. of rats	Nor	Adr	Dop.
		μg/kg/24 h				μg/kg/24 h				μg/kg/24 h		
		Controls 180 g + 22° C				Cold acclimated 345 g + 3° C				Cold acclimated 290 g + 3° C Adrenalectomized		
0	6	4 0	0 77	32	9	7 8	1.20	26	6	7 7	1 09	36
1	6	12 9	1 07	38	9	28.4	4.23	35	6	10 0	1.32	30
2	6	13 1	1 06	34	8	28 3	13 46	30	6	16 2	1.82	36
3	6	13 5	1 47	41	5	38.2	12 70	33	6	30 3	2 87	41
4	6	14 9	1 52	39	3	39 9	14 86	30	5	34 6	6 00	51
6	6	21 6	2 10	34	0	—	—	—	2	19 9	3 96	50
		Survival time >168 h				Survival time 69 h				Survival time 173 h		
		Warm acclimated 180 g + 3° C				Warm acclimated 310 g + 3° C				Warm acclimated 245 g + 3° C Adrenalectomized		
0	6	3 7	0 68	25	6	2 8	0 56	27	6	4 0	0 24	31
1	6	28 2	15 31	32	6	34 4	19 27	39	6	8 4	1 03	37
		Survival time 7 h				Survival time 17 h				Survival time 4 h		

adrenaline while the dopamine output increases by only 35 to 45 % The lower values found in adrenalectomized animals can possibly be due to the short period during which urine has been collected The excretion values for noradrenaline and adrenaline in cold acclimated rats were also unusually high In contrast, in adrenalectomized cold acclimated rats there was a gradual increase in the excretion of the three amines, and surprisingly these animals could live twice as long as intact cold acclimated ones

In organs of phenoxybenzamine treated rats at room temperature there was a marked depletion in both amines in heart, spleen and liver (Table XVI) In all groups of rats dead in the cold the noradrenaline depletion of organs was more pronounced than the one seen in the control group estimated at about the same time after the beginning of treatment The adrenaline concentrations, on the other hand, were about the same or higher The amine content of adrenal glands was very low in rats exposed to cold while no definite changes occurred in the control groups at room temperature The calculated resynthesis of adrenaline at the time of the death of animals in the cold was between 400 and 500 μg/kg/24 hours, which was higher than the maximal rate of resynthesis calculated in experiments at — 7° C (280—300 μg/kg/24 hours)

Table XIV. Catecholamine content of organs in warm- and cold-acclimated rats after ganglionic blockade Mecamylamine injected at a dose of 20 mg/kg s c every 3 hours for 12 hours Six rats per group

Groups	Adrenals		Heart		Spleen		Liver		Muscle	
	Nor	Adr	Nor	Adr	Nor	Adr	Nor	Adr	Nor	Adr
	µg/kg b wt		µg/g tissue		µg/g tissue		µg/g tissue		µg/g tissue	
Control group + 22° C No treatment	21 9	141	0 52	0 064	0 43	0 036	0 041	0 004	0 052	0 002
Mecamylamine + 22°C Killed 24 hours	22 8	119	0 45	0 075	0 40	0 053	0 038	0 005	0 043	0 004
Mecamylamine + 3° C Warm-acclimated Dead 10 hours	25 8	102	0 61	0 097	0 50	0 072	0 047	0 010	0 066	0 008
Mecamylamine + 3° C Cold acclimated Killed 24 hours	28 3	136	0 66	0 138	0 90	0 118	0 064	0 010	0 068	0 008

12 hours), the noradrenaline concentrations in all organs were not significantly changed but there was a depletion of adrenal glands in adrenaline and an increased content in other organs On the contrary, the noradrenaline concentrations in all organs of rats in the cold were high, especially in cold-acclimated animals Similarly, in all organs except adrenal glands, there was a large increase in the adrenaline content which was more conspicuous in cold-acclimated rats

Effect of adrenergic blockade on the catecholamine response to cold exposure

All rats in the cold died in hypothermia after adrenergic blockade with phenoxybenzamine (10 mg/kg/day) However, there was a significant difference in survival between warm- and cold-acclimated animals (*see* Table XV) Warm-acclimated rats could live for only a few hours in the cold and they became immediately hypothermic In contrast, cold-acclimated ones could live for a few days and their colonic temperature was normal till 24 to 36 hours before death occurred A higher dosage of phenoxybenzamine (20 mg/kg/day) did not shorten the survival time of cold-acclimated rats

As seen in Table XV, phenoxybenzamine largely increased the urinary excretion of noradrenaline in the control group at room temperature The adrenaline output gradually arose with prolongation of treatment but much less than noradrenaline Dopamine was not appreciably changed At all times the colonic temperature of these rats was normal Young and old warm-acclimated rats in the cold excrete very large amounts of noradrenaline and

Table XI Urinary excretion of catecholamines after adrenergic blockade produced by phenoxybenzamine in warm and cold acclimated rats in the cold at + 3° C compared to the control group maintained at room temperature The adrenolytic agent was used at a dose of 10 mg/kg day i p Day 0 indicates day before treatment

Days	No. of rats	Nor	Adr	Dop.	No of rats	Nor	Adr	Dop	No of rats	Nor	Adr	Dop
		μg kg 24 h				μg/kg/24 h				μg kg 24 h		
		Controls 180 g + 22° C				Cold acclimated 345 g + 3° C				Cold acclimated 290 g + 3° C Adrenalectomized		
0	6	4 0	0 77	32	9	7 8	1.20	26	6	7 7	1 09	36
1	6	12 9	1 07	38	9	28 4	4 23	35	6	10 0	1.32	30
2	6	13 1	1 06	34	8	29 3	13 46	30	6	16 2	1.82	36
3	6	13 5	1 47	41	5	38.2	12 70	33	6	30 3	2 87	41
4	6	14 9	1 52	39	3	39 9	14 86	30	5	34 6	6 00	51
6	6	21 6	2 10	34	0	—	—	—	2	19 9	3 96	50
		Survival time >168 h				Survival time 69 h				Survival time 173 h		
		Warm acclimated 180 g + 3° C				Warm acclimated 310 g + 3° C				Warm acclimated 245 g + 3° C Adrenalectomized		
0	6	3 7	0 68	25	6	2 8	0 56	27	6	4 0	0 24	31
1	6	28 2	15 31	32	6	34 4	19 27	39	6	8 4	1 08	37
		Survival time 7 h				Survival time 17 h				Survival time 4 h		

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Table XIV Catecholamine content of organs in warm- and cold-acclimated rats after ganglionic blockade Mecamylamine injected at a dose of 20 mg/kg s c every 3 hours for 12 hours Six rats per group

Groups	Adrenals		Heart		Spleen		Liver		Muscle	
	Nor	Adr	Nor	Adr	Nor	Adr	Nor	Adr	Nor	Adr
	µg/kg b wt		µg/g tissue		µg/g tissue		µg/g tissue		µg/g tissue	
Control group + 22 ° C No treatment	*21 9*	*141*	*0 52*	*0 064*	*0 43*	*0 036*	*0 041*	*0 001*	*0 052*	*0 002*
Mecamylamine + 22 °C Killed 24 hours	22 8	119	0 45	0 075	0 40	0 053	0 038	0 005	0 043	0 004
Mecamylamine + 3 ° C Warm acclimated Dead 10 hours	25 8	102	0 61	0 097	0 50	0 072	0 047	0 010	0 066	0 008
Mecamylamine + 3 ° C Cold acclimated Killed 24 hours	28 3	136	0 66	0 138	0 90	0 118	0 064	0 010	0 068	0 008

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Table XV Urinary excretion of catecholamines after adrenergic blockade produced by phenoxybenzamine in warm and cold-acclimated rats in the cold at + 3° C compared to the control group maintained at room temperature The adrenolytic agent was used at a dose of 10 mg/kg day i p Day 0 indicates day before treatment

Days	No of rats	Nor (µg/kg 24 h)	Adr	Dop	No of rats	Nor (µg/kg/24 h)	Adr	Dop	No of rats	Nor (µg/kg/24 h)	Adr	Dop
		Controls 180 g + 22° C				Cold acclimated 345 g + 3° C				Cold acclimated 290 g + 3° C Adrenalectomized		
0	6	4.0	0.77	32	9	7.8	1.20	26	6	7.7	1.09	36
1	6	12.9	1.07	38	9	28.4	4.23	35	6	10.0	1.32	30
2	6	13.1	1.06	34	8	28.3	13.46	30	6	16.2	1.82	36
3	6	13.5	1.47	41	5	38.2	12.70	33	6	30.3	2.87	41
4	6	14.9	1.52	39	3	39.9	14.86	30	5	34.6	6.00	51
6	6	21.6	2.10	34	0	—	—	—	2	19.9	3.96	50
		Survival time >168 h				Survival time 69 h				Survival time 173 h		
		Warm acclimated 180 g + 3° C				Warm-acclimated 310 g + 3° C				Warm-acclimated 245 g + 3° C Adrenalectomized		
0	6	3.7	0.68	25	6	2.8	0.56	27	6	4.0	0.24	31
1	6	28.2	15.31	32	6	34.4	19.27	39	6	8.4	1.03	37
		Survival time 7 h				Survival time 17 h				Survival time 4 h		

adrenaline while the dopamine output increases by only 35 to 45 %. The lower values found in adrenalectomized animals can possibly be due to the short period during which urine has been collected. The excretion values for noradrenaline and adrenaline in cold acclimated rats were also unusually high. In contrast, in adrenalectomized cold acclimated rats there was a gradual increase in the excretion of the three amines, and surprisingly these animals could live twice as long as intact cold acclimated ones.

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Table XVI Catecholamine content of organs in phenoxybenzamine treated rats at room temperature and in the cold Three rats per group at + 22° C and six at + 3° C

Groups	Adrenals		Heart		Spleen		Liver		Muscle	
	Nor	Adr	Nor	Adr	Nor	Adr	Nor	Adr	Nor	Adr
	μg/kg b wt		μg/g tissue		μg/g tissue		μg/g tissue		μg/g tissue	
Control + 22° C										
No treatment	29 8	150	0 61	0 067	0 49	0 027	0 040	0 003	0 061	0 003
Phenoxybenzamine + 22° C										
6 hours after inj	19 3	146	0 34	0 011	0 25	0 012	0 020	0 001	0 053	0 003
24 hours after inj	23 0	151	0 41	0 024	0 23	0 000	0 019	0 002	0 045	0 003
72 hours after inj	29 2	170	0 38	0 017	0 31	0 000	0 018	0 002	0 048	0 004
168 hours after inj	32 3	155	0 51	0 010	0 33	0 012	0 011	0 003	0 056	0 003
Phenoxybenzamine + 3° C										
Warm acclimated 180 g Survival time 7 h[1]	16 6	69	0 17	0 014	0 13	0 010	0 014	0 002	0 028	0 003
Warm acclimated 310 g Survival time 17 h[1]	10 7	42	0 14	0 021	0 09	0 008	0 011	0 003	0 018	0 002
Cold acclimated 345 g Survival time 69 h[1]	8 6	23	0 09	0 009	0 09	0 008	0 008	0 003	0 030	0 002
Adrenalectomized Warm acclimated 245 g Survival time 4 h[1]	—	—	0 24	0 031	0 11	0 040	0 011	0 003	0 054	0 006
Adrenalectomized Cold acclimated 290 g Survival time 173 h[1]	—	—	0 10	0 012	0 10	0 014	0 010	0 002	0 028	0 002

[1] Catecholamines estimated after the death of animals in the cold

Discussion

There is every reason to believe that noradrenaline is the sympathetic neurotransmitter, and that most of this hormone excreted in urine comes from the adrenergic nerve endings (*cf* EULER 1956, 1959) Our results on the output of noradrenaline in adrenalectomized rats exposed to cold are in full agreement with these conclusions The suprarenal contribution to the increased excretion of noradrenaline in rats exposed to + 3° C appears to be very small a finding also reported by LEBLANC and NADEAU (1961) However, it should be kept in mind that under more severe conditions the suprarenal contribution, as judged by the noradrenaline content of the glands, is more important as seen in chronic

experiments at $-7°$ C (*cf* Chapter IV) and after adrenergic blockade at $+3°$ C The noradrenaline suprarenal reserves, which are rather small, are used especially when a maximal secretion of noradrenaline is required

The adrenergic nerve origin of noradrenaline release by cold exposure is further demonstrated in experiments with mecamylamine At every dosage used the noradrenaline excretion is lower during ganglionic blockade than during the recovery period This is associated with a normal or elevated noradrenaline content of organs the first day of exposure or re exposure to cold, which is in contrast to our previous observations in normal warm- and cold-acclimated rats (*cf* Chapters III and IV) Therefore, it is likely that the reduced noradrenaline output is due to an inhibition of the release of the transmitter at the adrenergic nerve endings This conclusion is in agreement with that of SUNDIN (1958) who first reported a decreased response in the noradrenaline excretion induced by tilting after mecamylamine treatment in human The fairly large amounts of noradrenaline still excreted in urine in our experiments is due to an incomplete and intermittent blockade with the dosages and the time schedule used, as illustrated by measurements of the colonic temperature

The increased adrenaline excretion during and after mecamylamine is the result of a secretion from the adrenal glands, since it does not occur in adrenalectomized animals The lower adrenaline excretion values during the first 12-hour period show that ganglionic blockade under our experimental conditions effectively inhibited the suprarenal secretion, especially with a dose of 20 mg/kg, which is in accord with the results of MIRKIN (1958) and MARLEY and PATON (1961) It is unlikely that mecamylamine causes a direct secretion of adrenaline, as shown for tetraethylammonium (PATON and ZAIMIS 1952), since there *is no* relation between the excretion of adrenaline and the dose used It is more probable that the adrenaline secretion represents a compensatory mechanism for the reduced secretion of noradrenaline, especially in view of the inverse relationship between the excretion of noradrenaline and the adrenaline output during the second 12 hour period This further illustrated the supplementary role of adrenaline in the defense against cold

Most of the adrenaline secreted on exposure to cold comes from the adrenal medulla (*cf* also LEBLANC and NADEAU 1961), although adrenalectomized rats can still significantly increase their adrenaline excretion From these [illegible]
it can be [illegible]
to [illegible]
oc [illegible] Moreover the [illegible]
[illegible] extra adrenal sources

Whether the extra adrenal adrenaline comes from the adrenergic nerve endings or the chromaffin cells in the tissues is difficult to say By the use of appropriate testing techniques it could be shown that the active substance

released by adrenergic nerve stimulation conformed *in its properties with* noradrenaline, but most investigators have also stated that small amounts of adrenaline were simultaneously liberated (*cf* EULER 1959) In adrenalectomized animals exposed to cold the quantity of adrenaline excreted is small compared to the total amount of catecholamines, but it always represents about the same percentage of the total (3 to 6 %) This suggests that adrenaline is also released upon stimulation of the sympathetic nervous system by cold stimuli Of course, the release of an active substance on stimulation of the nerves does not necessarily mean that this substance is the corresponding chemotransmitter As reviewed by EULER (1959), *a great deal of evidence has accumulated showing that* adrenaline, even that released on stimulation of the adrenergic nerves, must have been located outside the adrenergic neurons and in all likelihood in the chromaffin cells The increase in the adrenaline excretion after histamine in adrenalectomized rats speaks in favor of this view, since this drug has been shown to act directly on the chromaffin cells (DE SCHAEPDRYVER 1959 a)

Dopamine has been found in the adrenal glands (GOODALL 1951, SHEPHERD and WEST 1953, DENGLER 1957) and in the sympathetic nerves (SCHÜMANN 1956) Neither adrenalectomy nor ganglionic blockade greatly affects the *urinary excretion of this amine, thus suggesting that most of the dopamine* found in urine comes from other tissues

The importance of catecholamines in the defense against cold is well illustrated in experiments with phenoxybenzamine Warm-acclimated as well as cold-acclimated rats can not withstand cold exposure after blockade of the physiological effects of catecholamines However, rats at room temperature do not become hypothermic and do not show visible shivering after phenoxybenzamine, suggesting that the blockade is not complete and that sufficient amounts of catecholamines can still reach the receptors and exert their actions to maintain *a normal body temperature in these conditions Pertinent to this is the ob*servation that tissues are hypersensitive to catecholamines after phenoxybenzamine (HOLZBAUER and VOGT 1954) Moreover, MOORE (1960) has reported that the calorigenic effects of adrenaline and noradrenaline in adult rats were enhanced after pre-treatment with phenoxybenzamine

The longer survival time of cold acclimated rats treated with phenoxybenzamine may be related to the increased sensitivity of these rats to adrenaline (RING 1942 HSIEH and CARLSON 1957 SWANSON 1957) and noradrenaline (HSIEH and CARLSON 1957, DEPOCAS 1960 a b) brought about by acclimation to cold *These experiments, however, can not give information* about the respective roles of adrenaline and noradrenaline since the blocking effect is not specific for one or the other amine Furthermore, it is impossible to attribute the effects of catecholamines specifically to their vasoconstrictor or metabolic action because phenoxybenzamine inhibits as well the blood pressure elevation as the hyperglycemia induced by adrenaline (HARVEY, WANG and NICKERSON 1952) In curarized cold acclimated rats HSIEH, CARLSON and GRAY (1957)

have shown that the adrenergic blocking agent piperoxane prevented the increase in oxygen consumption otherwise observed on exposure to cold This suggests that catecholamines act through their effects on metabolism

The effect of adrenalectomy on the survival of rats in the cold points to a more important role for noradrenaline than adrenaline Adrenalectomized animals can live in the cold at + 3° C, provided sufficient amounts of cortical hormones are supplied Moreover, as illustrated by the excretion of catecholamines on re exposure to cold, these rats can achieve some acclimation to cold Even without any adrenal tissue (HÉROUX 1955) or minimal doses of corticoids (DESMARAIS 1957), a certain degree of acclimation to cold, measured by the survival at lower environments, has been shown to be possible, provided animals were exposed to cold gradually and intermittently Attempts to artifically acclimate clipped rats to cold with adrenaline were unsuccessful (SELLERS, REICHMAN and THOMAS 1951) These results show that the adrenal medulla are not of great importance in the development of cold acclimation, and by inference point to the role of noradrenaline in this process

The increased sensitivity of cold acclimated rats to adrenaline (RING 1942, HSIEH and CARLSON 1957, SWANSON 1957) and especially noradrenaline (HSIEH and CARLSON 1957, DEPOCAS 1960 a, b), and the importance of an intact sympathetic nervous system for survival of rats in the cold (HSIEH, CARLSON and GRAY 1957) have been confirmed in the present study by the experiments with mecamylamine From these observations it is not possible, however, to correlate the protective action of catecholamines to their vasoconstrictor or their stimulating effect on metabolism Changes in either insulation or heat production can result in changes in body temperature, and it is well known that ganglionic blocking agents have a vasodilating effect In view of the weaker vasoconstrictor effect of noradrenaline on the skin vessels (*cf* EULER 1956), and the observation of HSIEH, CARLSON and GRAY (1957) that noradrenaline is more effective than adrenaline in preventing the fall in oxygen consumption caused by hexamethonium in cold acclimated curarized rats, it is more likely that the striking protective action of noradrenaline observed here is mediated by its metabolic effects

Adrenaline, but not noradrenaline, has a protective effect on the mecamylamine induced hypothermia in rats maintained at room temperature The metabolic actions of adrenaline in normal animals, such as increased oxygen uptake and hyperglycemia, have been repeatedly observed to be greater than those of noradrenaline (*cf* EULER 1956) An interesting finding has recently been reported by MOORE and UNDERWOOD (1960) In the newborn [illegible]

[illegible] ous observation that [illegible]
excrete fairly large amounts of noradrena [illegible]
fits in quite well with the finding of Moo [illegible]

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The importance of catecholamines in the defense against cold in warm- and cold acclimated rats was illustrated by the failure of animals to withstand cold exposure after blockade of the physiological effects of these amines

Adrenalectomized rats withstood moderate cold exposure, provided cortical hormones were supplied, but animals died very quickly after inhibition of the release of noradrenaline from the adrenergic nerve endings Moreover, in cold-acclimated rats noradrenaline was more effective than adrenaline in preventing the fall in colonic temperature caused by ganglionic blockade These results point to a primary role of noradrenaline in the response of rats to cold stress and in acclimation to cold They also add support to the hypothesis of adrenaline as a supplementary hormone in the defense against cold

It is quite generally accepted that shivering is the most important means of heat production in normal animals exposed to cold (*cf* CHATONNET 1959). An intriguing aspect of our results is the very rapid death of non-anesthetized, non-curarized warm-acclimated rats exposed to cold after ganglionic or adrenergic blockade, although shivering is very intense. Hypothermia and death may result from the inability of these animals to offset heat loss due to a reduction in insulation caused by the vasodilating effects of the drugs. However, in our experiments on clipped rats (*cf* Chapter VII), in which insulation was greatly reduced, the survival time was longer and shivering less intense. It seems therefore that some chemical thermogenesis is also present in warm-acclimated rats exposed to cold and that it can be blocked by mecamylamine and phenoxybenzamine. CANNON, QUERIDO, BRITTON and BRIGHT (1927) have already propounded the view that the secretion of catecholamines mediated part of the initial phase of metabolic increase during cold exposure, and that this contribution of chemical regulation was masked by early shivering. Recently, new evidence has been found (DAVIS, JOHNSTON, BELL and CREMER 1960) for the existence of a true chemical thermogenesis in addition to increased muscle tone and shivering in normal rats exposed to cold.

The results on the excretion of catecholamines in cold-exposed rats treated with phenoxybenzamine seem to contradict the observations on the limitations of the synthesis and/or secretion of catecholamines (*cf* Chapter IV). The excretion values of catecholamines are much higher than those previously found. This apparent contradiction is explained by the effect of phenoxybenzamine on the inactivation of catecholamines at/or near the receptor sites (BROWN and GILLESPIE 1957, BENFEY, LEDOUX and MELVILLE 1959, BENFEY, LEDOUX and SEGAL 1959, BENFEY 1961). An impairment in the destruction of amines will lead to a relative increase in the catecholamine excretion. Moreover, as observed by SCHAPIRO (1958), FURCHGOTT and KIRPEKAR (1960) and confirmed in the present study, phenoxybenzamine partially depletes organs of their catecholamines. Both an impaired inactivation and a release of catecholamines are then involved in the large excretion of adrenaline and noradrenaline caused by phenoxybenzamine. Surprisingly, however, we have noted that phenoxybenzamine did not affect the metabolism of injected catecholamines (*see* also AXELROD and TOMCHICK 1959), which might suggest different pathways for the inactivation of endogenous and exogenous catecholamines.

Summary

Catecholamine measurements in adrenalectomized and mecamylamine treated rats showed that the increased adrenaline excretion induced by cold exposure was mainly due to an increased secretion from the adrenal medulla while the increased noradrenaline output resulted from an increased release from the adrenergic nerve endings.

cold at + 3 C (36 animals) and the other maintained at + 22 C (36 animals) Catecholamines in urine and organs of six animals were followed at different times over a period of 42 days Thirty six animals of the same average weight were treated with an equivalent volume of reserpine solvent (citric acid benzyl alcohol and polyethylene glycol) Half of them were exposed to cold 48 hours after injection Eighteen rats without any treatment were used as absolute controls at + 22° C and + 3° C. Ten adrenal ectomized rats (184 g) receiving cortical hormones (2 mg of desoxycorticosterone and cortisone per rat per day) were reserpinized (0 25 mg kg subcutaneously) one week after operation and exposed to cold 48 hours after reserpine treatment This low dosage was used because of the high sensitivity of adrenalectomized rats to the effects of reserpine

Twelve cold acclimated rats (2 months at + 3° C 3?1 g) transferred to room temperature were injected with 5 mg/kg of reserpine or an equivalent volume of reserpine solvent (6 rats) and re exposed to cold 48 hours later Twelve cold acclimated animals (7 weeks at + 3° C, 3?0 g) were adrenalectomized at room temperature Two days after operation six rats were treated with 0 5 mg/kg of reserpine and six with the solvent The

was also exposed to + 3 C Six cold acclimated rats (2 months at + 3° C, 325 g) and six adrenalectomized cold acclimated ones (2 months at + 3° C 2?5 g) maintained in the cold room received a daily injection of 0 25 mg kg of reserpine subcutaneously All these groups were followed for a period of 30 days as regard to the catecholamines in urine and organs The colonic temperature was measured at frequent intervals during the course of these experiments

Results

Effect of a single dose of reserpine

Six to eighteen hours after a single large dose of reserpine (5 mg/kg) the colonic temperature remained between 34° and 35° C even in rats at room temperature If animals were exposed to cold immediately after injection they all died in hypothermia within 12 to 15 hours at + 3° C However, all rats exposed to the same temperature 48 hours after reserpine treatment survived and at all times their colonic temperature during exposure to cold was within the limits of normality

The catecholamine excretion pattern in reserpine treated rats at room temperature was largely altered (Fig 19) First there was a significant decrease in the noradrenaline output which persisted for at least three weeks Secondly adrenaline showed a marked increase which was maximal the first day after injection The adrenaline output decreased rapidly

e noradrenaline excretion than in intact animals (Fig 19) The output remained at about the same moderately elevated level for two weeks and then increased as high as in the control groups

CHAPTER VI

EFFECT OF RESERPINE ON THE CATECHOLAMINE RESPONSE TO COLD EXPOSURE

The results presented in the previous chapters clearly show that catecholamines are strongly involved in the defense against cold and in the acclimation process Blockade of the release or *of the physiological actions of these amines* effectively lowers the ability of warm- and cold-acclimated rats to withstand cold exposure (*cf* Chapter V) Survival of animals in the cold appears to be dependent on the capability of secreting in sufficient amounts biologically active catecholamines to maintain thermal balance An adequate secretion of these amines is, on the other hand, dependent upon the rate of synthesis and the availability of body reserves

Reserpine has been repeatedly shown to deplete adrenal glands and other tissues of their catecholamines (CARLSSON and HILLARP 1956, HOLZBAUER and VOGT 1956, CARLSSON, ROSENGREN, BERTLER and NILSSON 1957, DE SCHAEPDRYVER and PREZIOSI 1959 b) In some species, including rats, reserpine has a peripheral as well as a central action either on the adrenal glands (KRONEBERG and SCHÜMANN 1957, MIRKIN 1958 a, CALLINGHAM and MANN 1958 b) or on other organs (BRODIE, OLIN, KUNTZMAN and SHORE 1957, KARKI, PAASONEN and VANHAKARTANO 1959) The depletion caused by reserpine, at least in the adrenal medulla of cats and dogs, seems to be the result of a release and a secretion of amines (STJARNE and SCHAPIRO 1958, DE SCHAEPDRYVER 1959 a) This and the observation that reserpine does not apparently prevent a normal amine resynthesis (MIRKIN 1958 a, CALLINGHAM and MANN 1958 a) suggest that its peripheral action is on the storage mechanism Recent experiments on the uptake of catecholamines after reserpine treatment strongly point to this mode of action (BERTLER, ROSENGREN and ROSENGREN 1960 BERTLER, HILLARP and ROSENGREN 1960, HILLARP 1960, AXELROD, WHITBY and HERTTING 1961) *In vitro* experiments also speaks in favor of this view (EULER and LISHAJKO 1960, 1961 c)

The experiments described in the present chapter were undertaken to define the behaviour of rats exposed to cold after depletion of their catecholamine stores by reserpine.

Methods

Single dose of reserpine Seventy two rats with a mean weight of 176 g were injected subcutaneously with 5 mg/kg of reserpine (Serpasil, Ciba) in order to achieve satisfactory ... central depression ... for two ... osed to

Table XVII Catecholamine content of adrenal glands after a single injection of a large dose of reserpine (5 mg/kg s c) in rats maintained at room temperature or exposed to cold 48 hours after injection Day 0 represents the day before treatment and day 1 corresponds to the third day after injection or the first day in the cold at + 3° C Six rats at each time

Days	Reserpine + 22° C		Reserpine + 3° C	
	Noradrenaline	Adrenaline	Noradrenaline	Adrenaline
	μg/kg b wt., Mean ± S E.		μg/kg b wt., Mean ± S E.	
0	14.0 ± 1.7	11.9 ± 4.1	14.0 ± 1.7	11.9 ± 4.1
1	5.4 ± 0.8	6.3 ± 3.9	4.9 ± 0.7	5.3 ± 4.9
6	16.5 ± 1.4	13.1 ± 5.7	7.2 ± 1.0	9.1 ± 6.0
12	11.7 ± 1.1	12.0 ± 3.5	12.9 ± 1.2	14.6 ± 4.1
18	13.4 ± 0.9	11.6 ± 4.6	17.4 ± 1.3	15.0 ± 3.2
30	16.3 ± 0.8	12.2 ± 4.6	14.6 ± 1.2	15.3 ± 5.0
42	17.1 ± 1.2	13.7 ± 5.8	13.6 ± 0.9	14.1 ± 3.7

Table XVIII Adrenaline secretion and resynthesis in rats exposed to cold of + 3° C 48 hours after injection of a single large dose of reserpine (5 mg/kg s c) The first day in the cold corresponds to the third day after injection There were six rats at each time

Days in the cold + 3° C	Differences in the glands μg/kg b wt	Excretion in urine μg/kg/24 h	Adrenaline	
			Secretion μg/kg/24 h	Resynthesis μg/kg/24 h
Controls + 22° C	0	0.70	22	22
1	− 66	8.09	256	190
6	− 18	6.01	190	172
12	+ 27	3.61	111	138
18	+ 31	3.09	93	129
30	+ 34	2.11	67	101
42	+ 22	2.06	65	87
Controls + 33° C	0	0.56	17	17

noradrenaline output remained low There was also an increase in the dopamine excretion of reserpinized rats in the cold but proportionally less than in normal animals

The adrenal glands of reserpine treated rats at + 22° C were largely depleted of their catecholamines three days after injection, but the deficit was completely corrected five days later (Table XVII) In cold exposed rats the values were still low the sixth day in the cold Thereafter the noradrenaline content was normal while the adrenaline concentration was slightly above normal The resynthesis of adrenaline was largely accelerated in rats exposed to cold (Table XVIII) even during the first week when the glands were partially

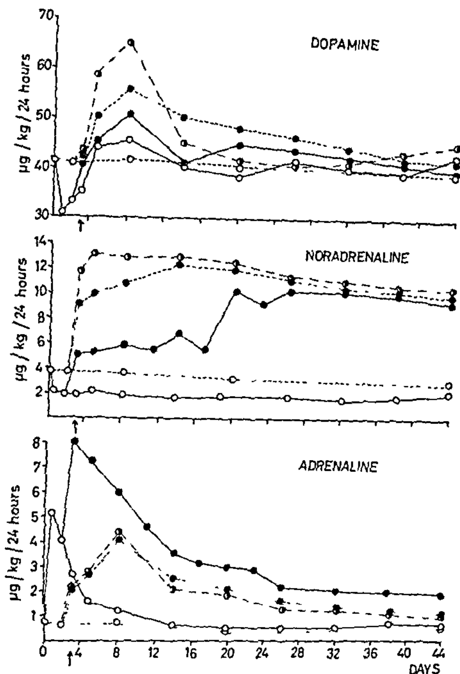


Fig 19 Effect of a single large dose of reserpine (5 mg/kg s c) on the urinary excretion of catecholamines (—) Reserpine treated rats at + 22° C (○) and + 3° C (●) ( ) Reserpine solvent treated rats at + 22° C ( ) and + 3°C (●) (- -) Controls at + 3° C (●) Arrow indicates third day after injection or first day in the cold Each point represents the mean of six rats

Adrenaline, which was in the descending phase at the time of exposure to cold, tremendously increased during the first day in the cold The excretion values for adrenaline were higher than those of the control animals as long as the

Table XIX Effect of a single dose of reserpine (0 25 mg/kg s c) on the catecholamine excretion of warm-acclimated adrenalectomized rats exposed to cold at + 3° C two days after treatment

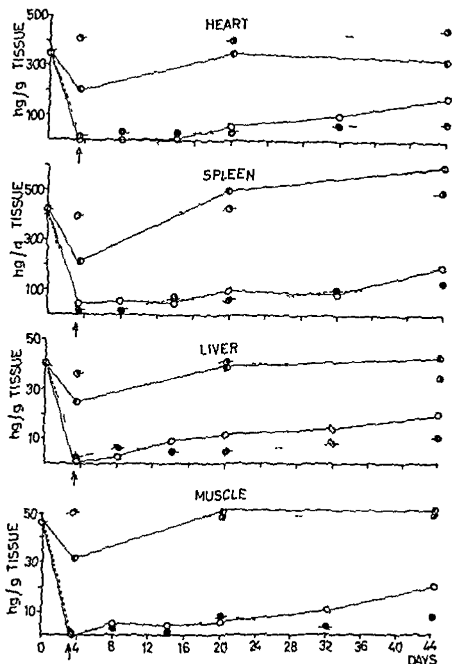
	Weight in g	No. of rats	Nor	Adr	Dop.
			µg/kg/24 h		
6th day after adrenalectomy + 22° C	183	10	4 4	0 12	37
2nd day after reserpine + 22° C	175	10	2 6	0 07	42
1st day + 3° C	167	10	7 3	0.59	41
2nd day + 3° C	174	5	8 2	0 68	58
4th day + 3° C	178	4	7 2	0 73	53
6th day + 3° C	182	2	6 1	0 74	46
12th day + 3° C	220	1	5 0	0 54	38

injection and 53 µg/kg/24 hours five days later Thereafter the adrenaline resynthesis was not accelerated

In other organs (heart, spleen, liver and skeletal muscle) there was a marked and prolonged depletion in the noradrenaline content (Fig 20) Forty four days after injection of a single large dose of reserpine the noradrenaline concentrations were only about one third of the normal values In rats exposed to cold there was a tendency to a more rapid repletion but the values were still far from normal Reserpine at room temperature caused a smaller (25 to 50 %) and shorter (2 weeks) depletion in the adrenaline content of heart, spleen and liver In cold exposed rats the adrenaline values were not significantly lower than in the control group in the cold except in skeletal muscle No dopamine could be detected in the spleen of reserpine and reserpine solvent treated rats in the cold Liver extracts prepared from the same groups of animals revealed equal amounts of dopamine in both the treated and control series There was no increase in the dopamine content of skeletal muscle in reserpinized animals exposed to cold while large amounts were found in the reserpine solvent group, as in normal cold exposed rats

Adrenalectomized rats were very sensitive to reserpine All animals treated with 1 mg/kg of reserpine died in hypothermia within 12 hours even at room temperature With a smaller dose (0.25 mg/kg) rats could survive, but their colonic temperature was diminished to about 34° C for several hours after injection They were, however, normothermic at the time of exposure to cold 48 hours after reserpine Five rats died during the first day in the cold, and only one out of ten has survived more than 6 days The average survival time for the nine rats dead in the cold was 61 hours

Table XIX shows the excretion of catecholamines in adrenalectomized reserpine treated rats The second day after treatment there was a decrease in the urinary excretion of both noradrenaline and adrenaline but no change in



n ra lrenal ne content of 3 C Controls at ind cates th rd day after

depleted However the level obtained was not higher than in intact animals in the cold (cf Table VII) in spite of a larger urinary excretion In rats at room temperature the resynthesis was also accelerated although much less than in cold exposed animals It was only 31 µg/kg/24 hours the third day after

Table XIX Effect of a single dose of reserpine (0 25 mg/kg s c) on the catecholamine excretion of warm-acclimated adrenalectomized rats exposed to cold at + 3° C two days after treatment

	Weight in g	No. of rats	Nor	Adr	Dop
			μg/kg/24 h		
6th day after adrenalectomy + 22°C	183	10	4 4	0 12	37
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[illegible]

er [illegible] second day after treatment there was a decrease in the urinary excretion of both noradrenaline and adrenaline but no change in

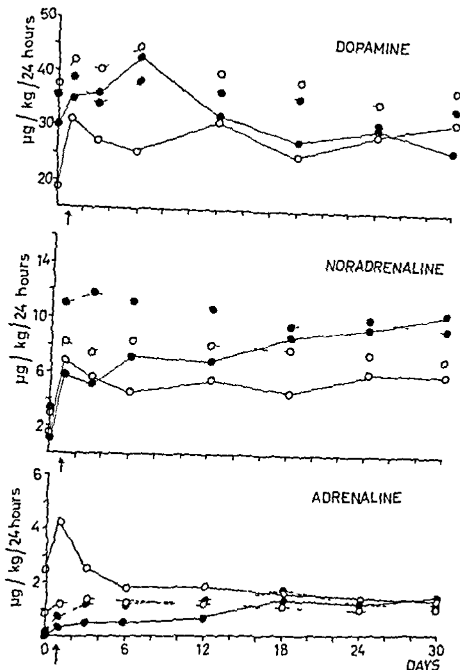


Fig 21 Effect of a s ngle dose of reserpine on the urinary excretion of catecholamines in intact (—) and adrenalectomized (●—) cold accl mated rats re exposed to + 3 C two days after ... erp ne sol ... nalectomi

the dopamine output, which was at variance with the results obtained in intact animals Exposure to cold increased the noradrenaline and adrenaline output although much less than in non treated adrenalectomized animals (cf Fig 16)

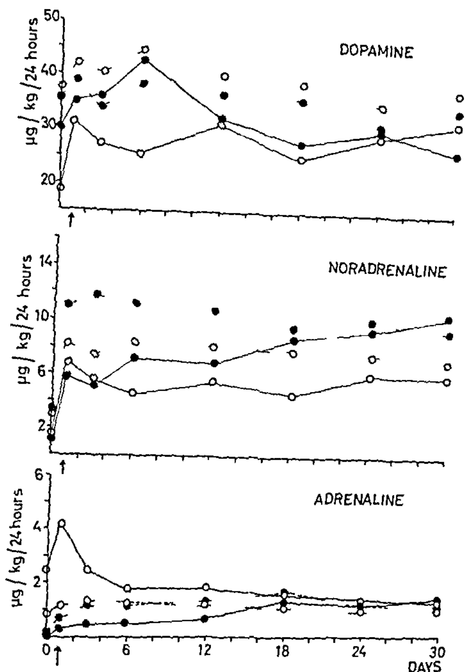
Surprisingly, the noradrenaline excretion values were approximately the same as those of intact rats injected with a large dose of reserpine Dopamine increased as much as in intact reserpinized animals but there was no initial drop in its output Estimations of catecholamines in organs of animals dead in the cold showed a depletion of about 90 % in the noradrenaline and 50 % in the adrenaline content

In cold acclimated rats the effects of a single dose of reserpine on the catecholamine excretion were quite similar to those observed in warm acclimated rats except that adrenaline showed a lower peak excretion and a faster decline thereafter (Fig 21) The colonic temperature was low (35° — 36° C) the first day after injection at room temperature but normal at all times during cold exposure The noradrenaline content of organs (heart, spleen, liver and skeletal muscle) at the end of the one month experimental period was markedly reduced (70—80 %) while the adrenaline content showed no significant change The concentration of adrenaline in adrenal glands was slightly elevated and the noradrenaline one normal

Reserpine reduced the excretion of both noradrenaline and adrenaline in cold acclimated adrenalectomized rats re exposed to cold (Fig 21) The values remained low for about two weeks and then increased to the control level There was no initial decrease in the dopamine output and an increased excretion was observed the sixth day The fairly large excretion values found in these two groups of cold acclimated rats may be due to the fact that adrenalectomy was performed after acclimation to cold No noticeable change in the colonic temperature of these rats was observed throughout the experiment Noradrenaline in organs of reserpinized animals was still not more than one fourth of the control values after one month of re exposure to cold

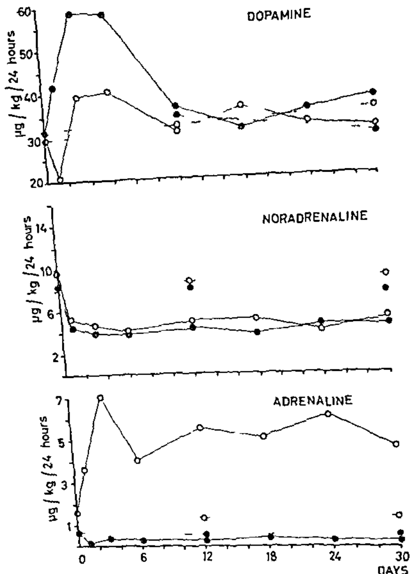
Effect of chronic administration of reserpine

A daily injection of a relatively small dose of reserpine (0 25 mg/kg) reduced the noradrenaline excretion in rats at room temperature or in the cold to the same level as a single large dose of the drug (Fig 22) In the present case, however this effect persisted as long as the treatment was continued Approximately the same values were obtained in animals treated with 0 1 mg/kg of reserpine The adrenaline excretion showed a slight, but significant, increase during the first 2 weeks of treatment in rats maintained at + 22° C Exposure to cold led to a larger increase in the adrenaline excretion The peak value on the sixth day was twice as high as in the control group and thereafter the values were maintained at a fairly high level With a dose of 0 1 mg/kg of reserpine the excretion of adrenaline in cold exposed rats reached a maximum of 6 35 μg/kg/24 hours the sixth day and remained around 4 00 μg/kg/24 hours thereafter In both groups of rats, at room temperature and in the cold, there was a decreased excretion of dopamine the first day after the beginning of treatment and an increased output of this amine the sixth day

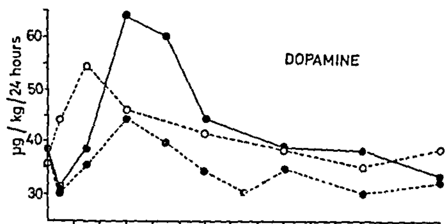


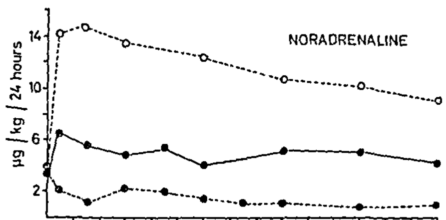
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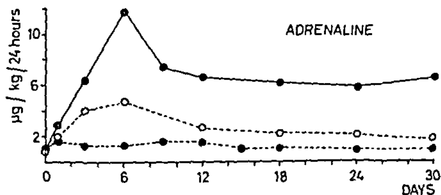
the dopamine output which was at variance with the results obtained in intact animals Exposure to cold increased the noradrenaline and adrenaline output although much less than in non treated adrenalectomized animals (*cf* Fig 16)



while adrenal ne showed small or no change In the adrenal glands noradrena line was 30 to 40 % lower than normally and adrenaline 10 to 20 % higher The colonic temperature was always within the normal range of variations







mean of six rats

Catecholamines in organs were estimated at 1, 12 and 30 days after the beginning of reserpine treatment in rats at room temperature. The noradrenaline content was found to be very low (10—20 % of the control values) at all times

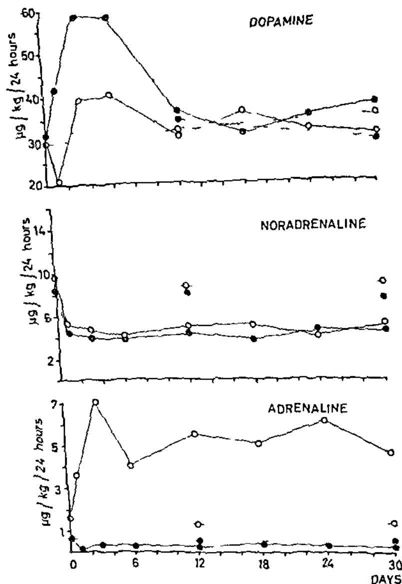


Fig 23

o mal range of variations

As seen in Fig 23, chronic administration of reserpine to intact and adrenalectomized cold-acclimated rats reduced the noradrenaline excretion by about 50 % and this effect persisted for the entire period of treatment in both groups of animals While reserpine largely increased the adrenaline output in intact animals, it decreased that of adrenalectomized rats The peak and steady-state levels for the adrenaline excretion in intact cold-acclimated rats were lower than those of intact warm-acclimated animals In both groups of cold-acclimated rats there was a maximum in the dopamine excretion 3 to 6 days after the beginning of treatment In the adrenalectomized group, however, dopamine did not decrease immediately after reserpine injection Organs were 75 to 90 % depleted of their noradrenaline in both groups of rats after one month of treatment In the adrenal glands of intact cold-acclimated rats the adrenaline concentration was high while the noradrenaline one was normal

Discussion

Although it is not directly related to the problem of cold exposure, the mode of action of reserpine should first be discussed in order to better understand the results obtained in rats exposed to cold A large number of papers have dealt with the action of reserpine on catecholamines, and marked discrepancies can be noted between the results of experiments conducted under more or less similar experimental conditions In discussing the influence of insulin on the catecholamine distribution in rats, BURN, HUTCHEON and PARKER (1960) pointed out that different strains of rats exhibited variable insulin sensitivity which also varied with seasonal and dietary conditions It is our feeling that the same reasoning applies to the action of reserpine on the excretion of catecholamines and the content of organs For instance, we have noted a greater sensitivity of the adrenal glands to the depleting effect of reserpine during the summer time than during the winter time Moreover, strain-dependent differences in the catecholamine loss of rat adrenals after reserpine administration have been observed by COUPLAND (1958) Care should therefore be taken in comparing the results from different workers

According to recent observations, it seems reasonable to assign the action of reserpine on catecholamines to an inhibition of the storage mechanism Pretreatment of animals with this drug impairs the uptake by adrenal medullary granules of newly formed catecholamines after dopa administration (BERTLER, HILLARP and ROSENGREN 1960, BERTLER, ROSENGREN and ROSENGREN 1960) or of administered noradrenaline and adrenaline by various tissues (DE SCHAEPDRYVER 1959 b, AXELROD, WHITBY and HERTTING 1961) The primary effect of reserpine, which is known to deplete the body stores of catecholamines, may be its interaction with the uptake of amines by storage sites Such an inhibition of the binding mechanism would possibly explain the depletion of organs by a release of the bound endogenous amines, but it would not account for the

decreased excretion of noradrenaline reported here and also by other workers (CARLSSON, ROSENGREN, BERTLER and NILSSON 1957, GADDUM, KRIVOY and LAVERTY 1958, CARLSSON, RASMUSSEN and KRISTJANSEN 1959)

From the observations that reserpine increases the rate of disappearance of administered adrenaline and noradrenaline in the body (AXELROD and TOMCHICK 1959, AXELROD 1960) and reduces the uptake of noradrenaline by various tissues (AXELROD, WHITBY and HERTTING 1961), these authors concluded that reserpine may exert its effects by preventing the binding of catecholamines thus exposing them to enzymic attack and more rapid metabolism Support to this view is given by the well demonstrated protective action of monoamine oxidase inhibitors on the catecholamine depletion caused by reserpine either in the adrenal glands or other organs (CARLSSON, ROSENGREN, BERTLER and NILSSON 1957, HOLTZ, BALZER and WESTERMANN 1957, SPECTOR, PROCKOP, SHORE and BRODIE 1957, DE SCHAEPDRYVER and PREZIOSI 1959 a) However, according to this scheme there should be an accumulation of amines outside the stores, presumably in the cytoplasmic sap, after inhibition of monoamine oxidase in reserpinized animals, which could not be confirmed in the rabbit brain by Weil Malherbe and Bone (1959) and in the rat brain by Green and Sawyer (1960) These results and our observations can better be explained by assuming that reserpine directly activates monoamine oxidase This assumption does not necessarily imply that monoamine oxidase is normally very active in the *inactivation* of catecholamines The action of reserpine may be either a liberation of the enzyme from some physical or chemical bond or an induction of *its* synthesis Also, reserpine can render conditions more favorable for enzymic attack of substrates

Preliminary experiments on the recovery of injected noradrenaline *in urine* of reserpinized rats strongly support this point of view Normal rats excreted 2 9 % of the injected dose as free noradrenaline *in 24 hours* and pre-treatment with iproniazid (100 mg/kg intraperitoneally 30 minutes before the injection of noradrenaline) slightly increased the yield (3 3 %) Animals treated with 0 25 mg/kg of reserpine for one week excreted only 1 6 % of the same dose of noradrenaline as free amine in 24 hours and pre treatment with iproniazid increased the recovery *to* 3 9 % These results suggest that the inactivation of noradrenaline is not only accelerated but more complete after reserpine, presumably by activation or *induction of* an inactivation enzyme Since this effect is corrected by iproniazid, and since reserpine does not activate the catechol O methyl transferase (AXELROD, WHITBY *and* HERTTING 1961), it is reasonable to assume that reserpine acts on the oxidative enzyme Discussion about the respective roles of monoamine oxidase *and* catechol O methyl transferase in the inactivation of catecholamines is beyond the scope of this paper

As judged from the *excretion values* of noradrenaline when corrected for the decreased recovery in urine, it appears that reserpinized animals excrete quite normal amounts of this amine, which suggests that under resting conditions the

synthesis and secretion of noradrenaline are neither inhibited nor accelerated by reserpine It also shows that the adrenergic transmitter can be produced and released immediately without being stored, since organs are almost completely depleted in noradrenaline

Perhaps the most puzzling aspect then is that along with a seemingly normal synthesis and secretion of noradrenaline there is a decreased adrenergic activity In reserpinized animals stimulation of the adrenergic system produces attenuated signs of sympathetic activity (CARLSSON, ROSENGREN, BERTLER and NILSSON 1957, MUSCHOLL and VOGT 1958, MIRKIN and CERVONI 1960), although there is no decrease in the sympathetic outflow from measurements of action potentials (IGGO and VOGT 1960) The biological effects of the transmitter released by stimulation of the adrenergic nerves will depend on the concentration attained at the receptor sites It is possible that the free amine which continues to be formed will now pour from its sites of synthesis and be almost completely metabolized due to the activation of monoamine oxidase The resulting level at the receptor sites may then be too low to produce a normal response If one assumes that the enzyme is held in the same neuron as noradrenaline, which is strongly suggested by the observations of HOLTZ and WESTERMANN (1956), much of the hormone formed in a neuron may be metabolized without even leaving the cell and exerting effects

In agreement with other workers (BRODIE, SPECTOR and SHORE 1959, BERTLER 1960, GREEN and SAWYER 1960), one can presume the existence of a dynamic steady-state relationship between the noradrenaline concentrations in the stored reserves and that in the soluble cytoplasm Normally, this steady-state is presumably maintained by a balance between the rate of synthesis and storage of the amine, and the rate of release and metabolism of the free amine Activation of metabolism by reserpine may result in a decrease in the free form which, through the operation of the principle of mass action, is adjusted by the release of a proportionate amount from the stored form When the stores are completely depleted, the newly synthetized material may be immediately and largely inactivated before leaving the cell Though this hypothesis can provide a good explanation for some experimental observations, it does not fit in too well with others It is probable that reserpine exerts an action as well on the storage as on the inactivation mechanisms, and that the two effects are additive

As to the adrenal medulla, on the other hand, it is necessary to distinguish between a direct peripheral action and an indirect central effect of reserpine (HOLTZ, BALZER and WESTERMANN 1957, DE SCHAEPDRYVER 1959 a) In the first case, there is a liberation of amines from their bound form followed by enzymatic destruction *in situ* which is subject to interference from monoamine oxidase inhibitors In the second case, there is a true secretion of catecholamines into the blood followed by the pharmacological effect inherent to the release of these amines Evidence for a true indirect secretory effect of reserpine

has been presented for rabbits (KRONEBERG and SCHÜMANN 1957, HOLTZ, BALZER and WESTERMANN 1957), dogs (DE SCHAEPDRYVER 1959 a) and cats (STJARNE and SCHAPIRO 1958) In rats the action of reserpine upon the suprarenals seems mainly a peripheral one Section of the splanchnic nerves or of the spinal cord at C_6 does not significantly reduce the adrenal depletion (KRONEBERG and SCHUMANN 1957, MIRKIN 1958 a, CALLINGHAM and MANN 1958 b) and iproniazid largely inhibits the reserpine induced depletion (HOLTZ, BALZER and WESTERMANN 1957, ZBINDEN and STUDER 1958, CAMANNI, LOSANA, MOLINATTI and OLIVETTI 1960) Some authors however, have obtained a large reduction in the depletion after denervation of the glands (ERÄNKÖ and HOPSU 1958 HILLARP 1960, CAMANNI, LOSANA, MOLINATTI and OLIVETTI 1960)

It is impossible to conclude from our experiments whether adrenaline is liberated ' or secreted ' or both from the adrenal glands after reserpine treatment Therefore, an accurate estimate of the adrenaline resynthesis from the excretion values in urine and the actual content of the glands according to the method previously described (*cf* Chapter III) is not feasible On one hand, if adrenaline is secreted as in normal conditions the urinary excretion represents 3 2 % of the amount secreted On the other hand, if adrenaline is liberated by reserpine, the urinary excretion represents then about 1 6% of the amount liberated, assuming that reserpine affects the quantity of adrenaline excreted as free amine to the same extent as noradrenaline In the first case, the calculated resynthesis of adrenaline the third day after injection of a single large dose of reserpine is 31 μg/kg/24 hours In the second case, it is 62 μg/kg/24 hours Even the greater value is not particularly high compared to those calculated in other experiments where the adrenal glands were also largely depleted in adrenaline (*cf* Chapter IV) This suggests some inhibition of the synthesizing capacity in reserpine treated rats The slow rate of adrenaline resynthesis suggested here can possibly account for the slow repletion of adrenal glands after reserpine treatment (*cf* also CALLINGHAM and MANN 1958 a, b, COUPLAND 1959 ERANKO and HOPSU 1961) This *finding is* at variance with the relatively rapid resynthesis reported after splanchnic stimulation (HÖKFELT and MCLEAN 1950, HOLLAND and SCHUMANN 1956, BYGDEMAN and EULER 1958)

Our results are in agreement with those of COUPLAND (1959) and ERÄNKO and HOPSU (1961) concerning the behaviour of [illegible]
of reserpine treated rats In the recover
not increase above normal in contrast
MANN (1958 a, b) It is difficult to find a pertinent explanation for this discrepancy

It is quite generally accepted that adrenaline and noradrenaline in the adrenal medulla are stored in separate granules present in special adrenaline and noradrenaline containing cells of the intact adrenal medulla (*cf* HAGEN and BARRNETT 1960, ERÄNKO 1960) There is also good evidence that practically

all the noradrenaline in organs is present *in the postganglionic fibers* while adrenaline is mostly located outside the adrenergic neurons, in all likelihood in chromaffin cells (*cf* EULER 1959). Moreover, EULER and HILLARP (1956) have shown that the adrenergic transmitter is partly stored in specific granules, which was confirmed by SCHÜMANN (1958) Using small doses of reserpine, some authors (ERÄNKÖ and HOPSU 1958, CAMANNI, LOSANA and MOLINATTI 1958) have reported a selective depletion of noradrenaline from the adrenal glands of rats Similarly, we have noted that reserpine depletes noradrenaline more readily than adrenaline. This and the observation that reserpine causes a greater and longer depletion of noradrenaline than adrenaline in heart, spleen and liver (*cf* also PAASONEN and KRAYER 1958) suggest a differential action of reserpine on the adrenaline and noradrenaline stores This might possibly be explained in terms of differences in accessibility of reserpine to the storage sites or of differences in innervation of the cells It is more likely, however, a matter of sensitivity of the storage granules to reserpine, since some quantitative differences have already been noted between the nerve granules and the adrenal medullary granules as regard to the *in vitro* releasing effects of temperature, hypotonic solutions, freezing and thawing and detergents (EULER and LISHAJKO 1961 a) Why the resynthesis of adrenaline seems to be accelerated but not that of noradrenaline after reserpine might be due to different locations of biosynthetic enzymes in the cells

Most of the dopamine excreted in urine comes from sources other than the adrenal glands and the adrenergic nerve endings, since adrenalectomy or ganglionic blockade does not significantly affect the excretion of this amine (*cf* Chapter V) Recently, FALCK, HILLARP and TORP (1959) have presented evidence for the storage of dopamine in a special type of chromaffin cells It is probable that reserpine liberates dopamine from these cells and its action is short-lasting as the one on the adrenal medullary chromaffin cells No explanation is at the present satisfactory for the different behaviour of adrenalectomized rats regarding the dopamine excretion after reserpine

The results obtained in reserpinized rats exposed to cold can now tentatively *be interpreted in the light of the preceding considerations* In all groups of animals studied, the noradrenaline excretion either immediately after a single

ships exist between the quantity of noradrenaline excreted in urine after injection as after secretion, it appears that these rats can synthesize and/or secrete about *10 µg/kg/24 hours of noradrenaline*, which is half the maximal secretion rate calculated from other experiments (*cf* Chapter IV) Reserpine seems therefore to interfere with the synthesis of noradrenaline, at least at high rates of resynthesis KIRSHNER (1957) has shown that the formation of noradrenaline from dopamine required the presence of the granular fraction, at least in the adrenal medulla Reserpine acting on the granules may possibly affect the

synthesis as well as the storage mechanism The constant maximal rate of noradrenaline secretion obtained in reserpinized animals may therefore represent the quantity of this amine which can be synthetized outside the granules, in the cytoplasmic sap and immediately released without being stored

The adrenaline released on exposure to cold presumably results from a true secretion from the adrenal glands directly into the blood stream Therefore, it is not subjected to the monoamine oxidase action and can exert its physiological effects If this is true it means that the adrenaline resynthesis is also inhibited by about 50 % after reserpine (*cf* Table XVIII) Even if adrenaline is believed to be formed in the cytoplasmic sap (KIRSHNER and GOODALL 1957), its synthesis can be indirectly reduced by the interference of reserpine with the synthesis of noradrenaline, its immediate precursor

It is difficult to say to what extent the noradrenaline released by cold stress in reserpinized animals is physiologically active In warm acclimated rats the steady state level in the noradrenaline excretion is less than half the excretion level observed in normal rats Therefore, the adrenaline secretion supplements the deficit and the excretion is about twice as high as in normal rats On the other hand in cold acclimated rats the corrected values for the noradrenaline excretion are about the same as in the control groups and yet the adrenaline output increases much more than in normal rats suggesting that the noradrenaline released is not as active as in intact animals That noradrenaline is not fully active is also shown by the high mortality of adrenalectomized rats ex [illegible]

[illegible] that the noradrenaline released is not, on the other hand completely inactive One should also keep in mind that reserpinized animals are more sensitive to adrenaline and noradrenaline, as first observed by BEIN (1953) and repeatedly confirmed by many workers

The striking reciprocal relationship between the curves for the noradrenaline and adrenaline excretion in rats exposed to cold after a single large dose of reserpine and the sustained high adrenaline excretion values in chronic experiments strongly confirmed the hypothesis of a supplementary role for adrenaline in the defense against cold The failure of non acclimated adrenalectomized rats to survive in the cold after reserpine treatment adds further evidence for this idea Recently HOFFMAN (1960) has also noted a complete loss of thermoregulation in adrenalectomized reserpinized animals exposed to cold with a certain degree of correction with adrenaline

The present results extend recent observations on the effect of reserpine in rats exposed to cold ZILBERSTEIN (1960) reported that rats injected with a large dose of reserpine and stressed immediately after injection had a lower ability to withstand cold exposure than intact animals DANDIYA, JOHNSON and SELLERS (1960) showed a rapid fall in oxygen consumption and colonic temperature of reserpine treated rats exposed to + 4° C Similar results were

obtained by TAYLOR (1961) These changes may be related to the release and depletion of adrenaline and noradrenaline produced by reserpine, as suggested by DANDIYA, JOHNSON and SELLERS (1960), but in our experiments rats exposed to cold two days after a large dose of reserpine survive and maintain thermal balance, *although the peripheral* organs are *still* largely depleted of their amines One can therefore wonder if the immediate effects of reserpine are solely due to its depleting action on the catecholamine stores

Summary

Acute or chronic treatment with reserpine led to a large noradrenaline depletion of organs associated with a reduced urinary excretion of this amine These effects are tentatively explained by a direct activation of a degradation enzyme, presumably monoamine oxidase Reserpine also caused a sharp increase in the adrenaline excretion owing to a release, direct and/or indirect, from the adrenal glands The reserpine-induced adrenaline depletion of organs was lesser and shorter than the noradrenaline one It is suggested that reserpine *exerts a differential action on the nerve storage granules and the chromaffin* storage granules

Indirect evidence is presented suggesting that reserpine inhibits to some extent the synthesis and/or secretion of catecholamines The observation that cold-exposed rats increase their noradrenaline excretion to the same moderate level irrespectively of the dose of reserpine used, the severity of the environmental conditions or the degree of cold acclimation points to a lower maximal synthesizing and/or secreting capacity in reserpine treated rats Similar conclusions can be drawn regarding adrenaline

The behaviour of reserpinized rats in the cold, interpreted in the light of these considerations, adds further support to the hypothesis that noradrenaline is primarily involved in the defense against cold while adrenaline represents a second line of defense

CHAPTER VII

LIMITATIONS OF ACCLIMATION EFFECTS OF STARVATION AND CLIPPAGE ON THE CATECHOLAMINE RESPONSE TO COLD EXPOSURE

Using the catecholamine excretion as a criterion, it has appeared that cold acclimation had its limitations (*cf* Chapter IV) This could be gathered from the failure of cold acclimated rats to maintain the same moderate levels of catecholamines on prolonged exposure to — 7° C and from the mortality, loss in weight and poor general condition of these animals Similar conclusions were drawn by HART, HEROUX and DEPOCAS (1956) from the persistence of muscle electrical activity in cold acclimated rats exposed to — 6° C These authors (DEPOCAS, HART and HEROUX 1957) pointed out that acclimation to cold extended the range of temperatures at which the initial response in heat production is adequate for temporary maintenance of thermal balance but with eventual hypothermia and death The breakdown in heat production mechanisms seems to be associated with a saturation of the synthesis and/or secretion mechanisms for catecholamines, as suggested by our observations in chronic experiments at — 7° C (*cf* Chapter IV)

It is likely, however, that acclimation to cold is also limited by factors other than environmental On one hand, it is well known that the metabolic response in animals exposed to cold is dependent on an adequate caloric intake (GIAJA and GELINEO 1934), and that starvation greatly influences the process of acclimation, as measured by the enhancement of survival at low temperatures (*cf* HART 1957) On the other hand, marked differences in mortality of rats exposed to cold were noted after insulation has been reduced by removal of the fur (SELLERS, YOU and THOMAS 1951) In view of the vasoconstrictor and metabolic actions of catecholamines, it was felt of interest to further investigate the process of acclimation to cold and its limitations in starved and clipped warm- and cold acclimated rats

Methods

Twelve warm acclimated rats (average weight 430 g) were deprived of food but allowed to drink water *ad libitum* Six of them were exposed to cold of + 3° C 24 hours after the beginning of starvation while six were kept at room temperature as controls and killed after 7 days of fasting for the estimation of catecholamines in organs Nine animals acclimated to + 3° C for 2 months (average weight 386 g) were transferred to room temperature, starved and re exposed to the same temperature 24 hours later Six adrenalectomized cold-

acclimated rats (6 weeks at + 3° C, 290 g) *maintained on cortical substitute* therapy (2 mg of desoxycorticosterone and cortisone per rat per day) were similarly treated To three rats in the cold-acclimated group, food was given back after 72 hours in the cold When rats died in the cold, organs were removed as soon as possible after death and frozen till the time of extraction If an animal died during the collection of urine, the excretion values for this rat were corrected for a 24-hour period The results reported for starved rats were calculated from the body weight at the beginning of experiments because of the important loss in weight caused by fasting Catecholamine concentrations in organs were also corrected for the weight loss due to starvation

Clippage was performed under light Nembutal anesthesia (35 mg/kg intraperitoneally) with an electric clipper Rats were clipped at night as completely as possible, the head excepted, and urine collection started the next morning to avoid the releasing effect of the anesthetic on catecholamines Animals were then exposed to cold 12 hours after clippage When necessary clippage was repeated once a week Twelve old warm-acclimated rats (average weight 310 g) were sheared and six of them exposed to cold 12 hours later, the six others being maintained at room temperature as controls This control group was exposed to cold after one month at + 22° C Six cold acclimated rats (2 months at + 3° C, 375 g) and six adrenalectomized cold-acclimated ones (6 weeks at + 3° C, 330 g) receiving corticoids (2 mg/rat/day) were transferred to room temperature, clipped and re-exposed to cold 12 hours later

Results

Effect of starvation on the catecholamine response to cold exposure

No significant difference was noted in the survival times of warm- and cold-acclimated starved animals exposed to cold Warm-acclimated rats lived on an average of 118 hours while cold-acclimated ones survived for 127 hours Adrenalectomized cold acclimated rats, however, died more quickly 73 hours All starved animals maintained at room temperature were still alive after seven days of fasting The colonic temperature of the controls at + 22 C was at all times normal but all rats in the cold were hypothermic for about 24 hours before death The body weight of starved rats at room temperature was diminished by 25 % at the end of the 7-day period of fasting The same relative decrease was observed in warm acclimated rats in the cold However the weight loss was slightly larger in cold acclimated animals (33 %)

Fasting at room temperature caused a three-fold increase in the adrenaline excretion whereas it slightly, but not significantly, decreased the noradrenaline output (Fig 24) The dopamine excretion dropped markedly during the first 24 hours and continued to decrease more slowly thereafter Warm-acclimated starved rats increase their noradrenaline excretion to maximal values the first day of exposure to cold The adrenaline excretion, on the contrary, gradually reached its maximum which coincided with the death of animals The dopamine

F g 24 Effect of prolonged s arva n on h rn

Table XX Catecholamine content of organs in starved and clipped rats Six rats per group

Groups	Adrenals		Heart		Spleen		Liver		Muscle	
	Nor	Adr	Nor	Adr	Nor	Adr	Nor	Adr	Nor	Adr
	μg/kg b wt		μg/g tissue		μg/g tissue		μg/g tissue		μg/g tissue	
Normal rats										
Warm acclimated + 22° C	25.8	153	0.55	0.064	0.47	0.038	0.041	0.004	0.055	0.003
Starved rats										
Warm acclimated + 22° C, 168 h	23.0	170	0.65	0.098	0.65	0.079	0.072	0.011	0.059	0.005
Warm acclimated, + 3° C 118 h[1]	14.0	63	0.48	0.050	0.52	0.064	0.046	0.013	0.046	0.009
Cold acclimated, + 3° C, 127 h[1]	28.2	100	0.70	0.084	1.01	0.243	0.096	0.021	0.064	0.011
Adrenalectomized Cold acclimated, + 3° C, 73 h[1]	—	—	0.34	0.036	0.44	0.041	0.028	0.002	0.036	0.001
Clipped rats										
Warm acclimated, + 3° C, 33 h[1]	12.2	76	0.27	0.020	0.38	0.068	0.029	0.007	0.014	0.004
Cold acclimated, + 3° C 30 days	21.3	159	0.51	0.018	0.57	0.057	0.044	0.005	0.058	0.005
Adrenalectomized Cold acclimated + 3 C 62 h[1]	—	—	0.14	0.017	0.20	0.061	0.028	0.008	0.022	0.003

[1] Catecholamines estimated after the death of animals in the cold

output was slightly higher than in the control group but still very low Cold-acclimated rats reacted quite similarly to warm-acclimated animals although the absolute excretion values were a little lower When food was given after three days in the cold, at which time both noradrenaline and adrenaline excretion figures seemed maximal and the dopamine output minimal, there was a return to the pre-fasting levels within 24 hours Adrenalectomized cold-acclimated starved rats excreted about the same amounts of noradrenaline and adrenaline as before starvation, but the dopamine output was largely reduced

The adrenaline content of organs (Table XX) was higher than normal after 7 days of fasting at room temperature There was also an increase in the noradrenaline concentrations of heart, spleen and liver In rats exposed to cold, the noradrenaline concentrations were lower than in the starved group at + 22 C

in warm acclimated rats and higher in cold acclimated ones The adrenal glands were depleted of their adrenaline in both groups of animals The re synthesis of this amine attained 307 μg/kg/24 hours in the warm acclimated group and 280 μg/kg/24 hours in the cold acclimated one These values were identical to those found in chronic experiments at — 7° C (cf Chapter IV) and they were considered as the limit for the adrenaline synthesis In other organs of cold exposed rats especially cold acclimated ones the adrenaline figures were largely elevated A decreased amine content was found in organs of adrenalectomized cold acclimated rats

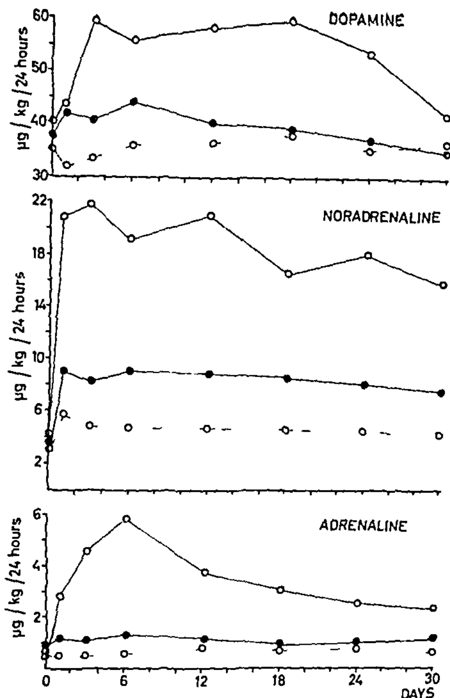
Effect of cl ppage on the catecholamine response to cold exposure

A striking difference in the survival of clipped rats exposed to cold was observed Warm acclimated animals could live for only one day in the cold (average 33 hours) while cold acclimated ones were all alive one month after re exposure Clipped rats maintained at room temperature for one month as controls and then exposed to cold lived on an average of 75 hours

The colonic temperature of clipped rats at room temperature was usually 0 5° to 0 7° C lower than normal Cold acclimated rats in the cold maintained a normal colonic temperature for the one month experimental period while others died quickly in hypothermia Rats lost weight during the first and second weeks after clippage The weight loss was more pronounced in cold acclimated rats in the cold (20 %) than in warm acclimated ones at room temperature (10 %)

Clippage significantly raised the noradrenaline excretion of rats maintained at + 22° C This effect was more evident during the first week but still present after one month (Fig 25) Adrenaline and dopamine did not show any significant changes Although clipped rats lost weight during the first week after clippage, it was not sufficient to explain the increased noradrenaline excretion since it persisted thereafter while the growth proceeded regularly

Cold acclimated clipped rats lived in the cold but as seen in Fig 25 they reacted much more intensively than intact cold acclimated animals re exposed to cold The noradrenaline excretion was maximal the first week of re exposure and slowly decreased with time while the adrenaline output showed a definite peak after one week in the cold Dopamine was excreted in large amounts for a long period of time The high values for adrenaline and noradrenaline after one month of re exposure could partially be explained by the 20 per cent weight loss during exposure to cold The corrected figures however, were still significantly increased over those of intact cold acclimated rats re exposed to cold Adrenalectomized cold acclimated clipped rats lived on an average of 78 hours on re exposure to cold and the *excretion values for n* [illegible]
and [illegible]
[illegible]
[illegible]



F g 25 Effect of cl ppage on tl e ur nary excre on of ca cel olam nes n arm accl mated rats (○) ma nta ned at + 22 C and n cold accl mated rats (○— re exposed to 3 ((●) Uncl pped cold accl mated ra s re exposed to cold S x ra s per group

Warm acclimated clipped rats exposed to cold excreted immediately maxi mal amounts of catecholamines 18 9 μg/kg/24 hours for noradrenaline and 12 80 μg/kg/24 hours for adrenaline during the first day On the contrary,

warm acclimated animals kept at room temperature for one month after clippage showed maximal excretion values for noradrenaline the first day of exposure to cold, but adrenaline reached its maximum only the third day before death occurred At that time the adrenaline output was 11 68 μg/kg/24 hours, which means a secretion of 374 μg/kg/24 hours and a resynthesis of 258 μg/kg/24 hours since the adrenaline content of adrenals was 37 μg/kg of body weight compared to 153 μg/kg of body weight in the control group

In all organs there was a depletion of noradrenaline in warm acclimated rats dead in the cold (Table XX) However, normal concentrations of amines were found in organs of cold acclimated clipped rats killed after one month of re exposure to cold The resynthesis of adrenaline in warm acclimated clipped animals was 310 μg/kg/24 hours, as measured from the excretion value of this amine during the first day in the cold and the content of adrenals removed immediately after death

Discussion

Small differences in the survival time and the excretion of catecholamines were noted between starved warm- and cold acclimated rats on exposure to cold, a result which is at variance with what we have seen up till now Many workers have compared the survival of warm- and cold acclimated animals at low temperatures when deprived of food (SEALANDER 1951, 1953, HART 1953, 1957) While differences in the anticipated direction were present, they were very small compared to those found in animals fed *ad libitum* BAKER (1960) also reported that the survival time of cold acclimated rats exposed to cold of + 2° C
ones
preve
hours

These results are not surprising It is likely a question of energy reserves of cold acclimated animals which are usually smaller than those of warm acclimated animals of the same age Glycogen concentrations are smaller in heart, liver, diaphragm and fat from perirenal and interscapular regions of the rat after 45 days at 1° — 3° C (BAKER and SELLERS 1953, VAUGHAN, HANNON and VAUGHAN 1958) Total body fat is also much lower in cold acclimated rats (PAGÉ and BABINEAU 1953, YOUNG and COOK 1955) It is therefore conceivable that cold acclimated starved rats can not withstand cold exposure for a longer time than warm acclimated ones of about the same age since the
reserves
these rat
in anim. (SELLERS, REICHMAN, THOMAS and YOU 1951, COTTLE and CARLSON 1954 HART, HEROUX and DEPOCAS 1956, HEROUX, HART and DEPOCAS 1956) Under such conditions the more rapid depletion of

energy reserves in cold-acclimated animals would offset any advantage of increased thermogenic capacity

The sensitivity of cold-acclimated rats to the metabolic effects of catecholamines (RING 1942, HSIEH and CARLSON 1957, DEPOCAS 1960 a, b) may account for the observations that fasting cold-acclimated rats maintain blood sugar levels as well as do fasting warm-acclimated rats (HANNON and YOUNG 1959) and higher fasting liver glycogen stores than controls (PAGE and BABINEAU 1954, PAGE, BABINEAU and LACHANCE 1955, FELTS and MASORO 1959) with a lower or equivalent secretion of catecholamines Insulin hypersensitivity was also observed in the cold-acclimated rat (BAKER and SELLERS 1953) The insulin and noradrenaline effects illustrate increased metabolic sensitivity of the cold-acclimated rat, presumably in terms of substrate mobilization Worthy to note is that starvation does not appreciably change the catecholamine concentration of skeletal muscle in rats exposed to cold, and the glycogen level in this tissue is not changed by acclimation (BAKER and SELLERS 1953, VAUGHAN, HANNON and VAUGHAN 1958).

The importance of an adequate food intake in rats exposed to cold is well illustrated by the rapid return of the excretion values of catecholamines to the pre-fasting levels when food is given back It also shows that acclimation to cold was only temporarily affected by starvation, but by no means lost The high excretion of catecholamines can then be regarded as a superimposed secretion due to the severity of conditions

The low excretion of dopamine in starved rats emphasizes the dependence *of this amine on the immediate precursors present in the diet for its formation* (*cf* also Chapter II) Furthermore, since the noradrenaline and adrenaline excretion can still be maximal with a low output of dopamine, it can be infered *that dopamine is not only a precursor of other amines, but also an end product* in the biogenesis of catecholamines

It has been proposed that the introduction of the side-chain hydroxyl group *is the rate-limiting step in the noradrenaline formation* (*cf* BLASCHKO 1959) The results obtained in starved rats at room temperature are not incompatible with that view But they also show that hydroxylation may proceed very rapidly when starved animals are exposed to cold *Indeed, the synthesis of* noradrenaline and adrenaline can be maximal without marked accumulation of dopamine This suggests a facilitation of the rate-limiting step by cold stimuli

A dramatic difference in the survival of warm- and *cold-acclimated clipped* rats was observed, a finding previously reported by SELLERS, YOU and THOMAS (1951) This is associated with large differences in the catecholamine excretion It appears that, in warm-acclimated rats, *the synthesis and/or secretion of both* noradrenaline and adrenaline being maximal the first day of exposure, animals die in hypothermia very quickly because of the immediate saturation of the catecholamine synthesis and/or secretion *mechanisms On the contrary, owing* to an increased sensitivity of cold-acclimated rats to catecholamines, there is still

a good margin of security in the adrenaline synthesis and/or secretion in these animals, and consequently they can survive in the cold

Strikingly, the catecholamine excretion pattern of cold acclimated clipped rats resembles the one observed in intact warm acclimated animals exposed to cold for the first time This is apparently in contradiction with what we have considered as a sign of acclimation to cold using the catecholamine excretion as a criterion It is believed that, in fact, these rats undergo acclimation to a new level Conditions have been changed and these animals are probably in a worse situation than intact non acclimated rats It is generally recognized that cold acclimation does not bring about an increase in overall insulation but rather a reduction (*cf* HART 1957) On one hand, there is an increased peripheral blood flow and consequently an increased peripheral heating, presumably due to vasodilatation of the vessels On the other hand, it is unlikely that there is a thickening of subcutaneous deposits of fat since the total body fat is much lower in cold acclimated rats Therefore, the insulating value of subcutaneous tissues is reduced and clipped cold acclimated rats should overcome a greater heat loss than intact animals exposed to cold The increase sensitivity of cold acclimated rats to catecholamines would then allow development of acclimation to cold otherwise impossible in normal rats, under such conditions which largely increase the catecholamine requirements Cold-acclimated rats also have the advantage that muscular activity does *not* eliminate non shivering thermogenesis, with the result that the total heat production is sufficient to offset the fall in insulation, and body temperature is maintained

SELLERS, REICHMAN, THOMAS and YOU (1951) have studied the effect of clippage on the metabolism of rats exposed to cold 3 hours after recovery from anesthesia They reported that the level of oxygen consumption reached by clipped non acclimated animals was on the average 30 per cent higher than that of unclipped animals This may be related to the higher adrenaline secretion reported here They also found that clipped cold acclimated rats increased their rate of oxygen consumption about 40 per cent above the levels observed before clipping or in intact animals exposed to cold, which is pertinent to the increased sensitivity of cold acclimated rats to catecholamines, since the catecholamine secretion does not raise more than in normal rats

Summary

Warm- and cold acclimated rats could not withstand prolonged exposure to cold after restriction of food Their catecholamine excretion rapidly attained maximal values and animals died in hypothermia The same happened with warm acclimated rats whose insulation has been reduced by removal of the fur Cold acclimated clipped rats survived in the cold, but their catecholamine response was much more intense than that of intact cold acclimated animals

These two series of experiments strongly support the view that acclimation to cold is limited by the finite capacity of the organism to produce and/or secrete sufficient amounts of catecholamines to maintain thermal balance They also emphasize the necessity for cold acclimated animals to reach a new level of acclimation when conditions are changed The previous degree of acclimation increases the chances to attain that new state of equilibrium by virtue of an increased sensitivity of cold acclimated rats to catecholamines, thus extending the time at which the catecholamine production and/or secretion becomes saturated Finally, the importance of adrenaline as a supplementary hormone of defense against cold is shown by the lower ability of adrenalectomized corticoids maintained rats to support cold exposure under severe conditions

GENERAL CONCLUSIONS

The results of the present study on the production and release of catecholamines in response to acute and chronic exposure to cold indicate that these amines are strongly involved in the defense against cold and in the acclimation process Blockade of the release or of the physiological effects of catecholamines renders warm and cold acclimated rats unable to withstand cold exposure Thus survival of animals in the cold appears to be dependent upon the capability of the organism to secrete sufficient amounts of biologically active amines to maintain thermal balance

The evidence obtained indicates that noradrenaline is the main mediator in the chemical regulation of heat production The hypothesis is put forward that adrenaline acts as a second line of defense against cold which supplements the readily limited synthesis and/or secretion of noradrenaline The adrenaline response is secondary timewise but of importance, since it can make the difference between survival and death of animals in the cold when the conditions become more severe

Since the mechanisms regulating the catecholamine synthesis and/or secretion are limited the resistance of animals to cold exposure is related to the finite capacity of the organism to synthesize and/or secrete noradrenaline and adrenaline

Acclimation to cold leads to an increased sensitivity of the tissues to the calorigenic effects of catecholamines, especially noradrenaline, thus exerting a sparing action on the production and/or secretion of catecholamines The striking effect of cold acclimation seems to be an extension of the time at which the mechanisms for the synthesis and/or secretion of noradrenaline and adrenaline become saturated Consequently it enhances survival at a lower environmental temperature

Cold acclimation is also limited in the sense that acclimation under certain conditions does not necessarily allow acclimation under other conditions The previous degree of acclimation, however, increases the probability of attaining a new level of equilibrium by virtue of increasing the sensitivity to catecholamines

Noradrenaline released as a result of cold exposure chiefly originates from the adrenergic nerve endings while adrenaline is secreted mainly from the adrenal glands Neither the site of origin nor the exact role of dopamine in the response to cold stress is at the present clear

These two series of experiments strongly support the view that acclimation to cold is limited by the finite capacity of the organism to produce and/or secrete sufficient amounts of catecholamines to maintain thermal balance They also emphasize the necessity for cold-acclimated animals to reach a new level of acclimation when conditions are changed The previous degree of acclimation increases the chances to attain that new state of equilibrium by virtue of an increased sensitivity of cold-acclimated rats to catecholamines, thus extending the time at which the catecholamine production and/or secretion becomes saturated Finally, the importance of adrenaline as a supplementary hormone of defense against cold is shown by the lower ability of adrenalectomized corticoids maintained rats to support cold exposure under severe conditions

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WITH SPECIAL REFERENCE TO SYMPATHO-INHIBITORY MECHANISMS

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Introduction

Neurophysiological research is becoming concerned to a steadily increasing extent with the functional organization of the cerebral cortex with its dependence on the complex afferent input from a variety of receptors and with the principles as to how integrated messages are sent to the effector cells of the organism via some of its three main efferent routes the somatomotor system, the visceromotor system and the neurohormonal system With special regard to the organization of the efferent systems the great majority of studies has so far been devoted to the somatomotor system and hence our knowledge of the central nervous control of the skeletal muscles is comparatively good

In contrast little is at present known about the central control of the autonomic system particularly as regards its more detailed organization and in some respects even the basic features of this control are poorly understood It is however an everyday experience that emotionally initiated reactions like fright rage mental stress etc — physiological phenomena generally considered to emanate from cortical levels — are accompanied also by characteristic promptly occurring autonomic reactions including cardiovascular adaptations Mental stress for example is often associated with vasomotor adjustments such as pallor of the skin embarrassment and shame with blushing Rage and fear usually induce cardiac acceleration and blood pressure elevation and very dramatic cardiovascular changes in the opposite direction are known to occur in the emotional fainting reaction Thus centrally induced autonomic reaction patterns of both excitatory and *inhibitory types* are *readily identified* when they become very pronounced There is however, much to indicate that such easily recognized dramatic reactions are not isolated phenomena but are indeed especially obvious manifestations of more or less steadily operating central visceromotor adjustments This *can be* exemplified by the changes in heart rate *accompanying shifts* in alertness reactions emanating from the highly organized nervous structures generally considered to be *responsible* for the mental processes

Current views concerning the cortical influence on the autonomic nervous system are apart from observations of the above mentioned type *mainly*

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blood flow changes can occur with only minor changes in mean blood pressure Similarly, experimental activations of the hypothalamic vasodilator fibre 'centre', inducing drastic cardiovascular adjustments often leave arterial blood pressure almost unchanged (ELIASSON *et al* 1951) Arterial blood pressure recordings are only able to indicate whether an elicited cardiovascular response pattern is predominantly excitatory or inhibitory with regard to *e g* sympathetic vasoconstrictor fibre activity, and do not reveal regional differences in blood flow variations induced

Nevertheless the studies so far carried out have been of paramount importance in the initial identification of cortical structures which exert an influence on the cardiovascular system and in indicating whether this influence is essentially excitatory or inhibitory It is however, not within the scope of the present investigation to present a comprehensive review of such studies The reader is for this purpose referred to recently published reviews by FOLKOW (1955 1956) RUSHMER and SMITH (1959), DELGADO (1960) and UVNÄS (1960) Only those studies having a more direct bearing on the present topic will be discussed in more detail, notably those dealing with the limbic system and its influence on the circulation

Cortical areas from which cardiovascular effects can be elicited appear to be restricted to anterior parts of the brain They include the motor and premotor cortex the anterior parts of the temporal lobes and certain sections of the limbic system like the anterior parts of the cingulate gyrus, the subcallosal gyrus and the insula Although there is general agreement as to the location of cortical structures influencing the cardiovascular system, the detailed organization of the responses obtainable when they are stimulated is with few exceptions unknown and their functional significance little understood This appears especially true of cardiovascular changes elicited from anterior parts of the limbic system as will be discussed more in detail below Those studies which have been performed with techniques allowing quantitative evaluations of cortically induced neurogenic adjustments of the cardiovascular system have mostly dealt with the motor and premotor cortex or with structures of special importance for the control of cutaneous blood flow They are, however of particular interest as they reveal that cortical mechanisms can induce complex cardiovascular changes, sometimes with highly selective engagements of certain functionally separated vascular sections (see *e g* GREEN and HOFF 1936, 1937, LUND 1943, STRÖM 1950 1960 ELIASSON and STRÖM 1950 ELIASSON, LINDGREN and UVNÄS 1952) Even in such studies it has sometimes been difficult to ascertain to what extent the observed effects were essentially cortical in origin or merely the result of secondary adjustments from the different types of cardiovascular receptors, and/or caused by

based on experiments where the effects of stimulation and ablation of cortical regions have been observed In this way a number of autonomically innervated structures has been shown to possess a cortical repre entation Thus topical stimulations of cortical structures have *beside cardiovascular changes* produced contractions of the nictitating membrane pupillary changes saliva tion changes in depth and rate of respiration alterations of gastrointestinal motility and secretion piloerection sweating and increased tone of the urinary bladder In most *previous studies only* few parameters have however been recorded simultaneously and only exceptionally have techniques been u ed which allow an analysis of the elicited responses in terms of their quantitative interrelationships Accordingly with few exceptions the organization of the *response patterns and their functional* significance are at pre ent largely unknown

The cortical influence on the *cardiovascular system* is not exceptional in this respect a fact that was recently emphasized by the organization of a conference especially devoted to central nervous system control of circulation (Physiol Rev 1960 *40* Suppl 4) In the orientation of this conference the opinion was expressed that an important reason for the di crepancy between our present knowledge of the central control of the somatomotor system as compared with the visceromotor control of *e g* the cardiovascular system appears to be the fact that comparatively few neurophysiologists have devoted their interest to the autonomic nervous system and its influence on subordi nate effector systems On the other hand relatively few scientists specializing in cardiovascular research have directed their attention to the central nervous control of the circulation

On the whole the present state of knowledge of the cortical influence on the circulation is with few exceptions still largely at an exploratory stage Probably this is partly due to the considerable technical *difficulties inherent* in experiments directed to more exact analyses of the central nervous control of the circulation Most previous studies have generally been concentrated on the question whether and if so to what extent stimulation within different cortical areas affects some easily recorded parameters *like arterial blood* pressure and heart rate Arterial blood pressure alone is however often a very poor indicator of peripheral vascular reaction patterns especially when differential adjustments take place under such circumstances blood pressure *is often very little affected Even a pronounced* vasodilatation within one region can be completely balanced by vasoconstriction within other tissues and or by an increased cardiac output leaving blood pressure practically unchanged Under both physiological and experimental conditions this is often the case During muscular exercise for example extensive regional

blood flow changes can occur with only minor changes in mean blood pressure Similarly experimental activations of the hypothalamic vasodilator fibre centre inducing drastic cardiovascular adjustments often leave arterial blood pressure almost unchanged (ELIASSON *et al* 1951) Arterial blood pressure recordings are only able to indicate whether an elicited cardiovascular response pattern is predominantly excitatory or inhibitory with regard to *e g* sympathetic vasoconstrictor fibre activity and do not reveal regional differences in blood flow variations induced

Nevertheless the studies so far carried out have been of paramount importance in the initial identification of cortical structures which exert an influence on the cardiovascular system and in indicating whether this influence is essentially excitatory or inhibitory It is however not within the scope of the present investigation to present a comprehensive review of such studies The reader is for this purpose referred to recently published reviews by FOLKOW (1955 1956) RUSHMER and SMITH (1959) DELGADO (1960) and UVNÄS (1960) Only those studies having a more direct bearing on the present topic will be discussed in more detail notably those dealing with the limbic system and its influence on the circulation

Cortical areas from which cardiovascular effects can be elicited appear to be restricted to anterior parts of the brain They include the motor and premotor cortex the anterior parts of the temporal lobes and certain sections of the limbic system like the anterior parts of the cingulate gyrus the subcallosal gyrus and the insula Although there is general agreement as to the location of cortical structures influencing the cardiovascular system the detailed organization of the responses obtainable when they are stimulated is with few exceptions unknown and their functional significance little understood This appears especially true of cardiovascular changes elicited from anterior parts of the limbic system as will be discussed more in detail below Those studies which have been performed with techniques allowing *quantitative* evaluations of cortically induced neurogenic adjustments of the cardiovascular system have mostly dealt with the motor and premotor cortex or with structures of special importance for the control of cutaneous blood flow They are, however of particular interest as they reveal that cortical mechanisms can induce complex cardiovascular changes sometimes with highly selective engagements of certain functionally separated vascular sections (see *e g* GREEN and HOFF 1936 1937 LUND 1943 STRÖM 1950 1960 ELIASSON and STRÖM 1950 ELIASSON LINDGREN and UVNÄS 1952) Even in such studies it has sometimes been difficult to ascertain to what extent the observed effects were essentially cortical in origin or merely the result of secondary adjustments from the different types of cardiovascular receptors, and/or caused by

'autoregulatory' flow adjustments Accordingly, it is desirable that the cortically elicited responses should not be examined without simultaneous consideration of the extent to which they are modified by reflexes emanating from the cardiovascular receptors or by local changes in vascular tone

Further, these relatively few more detailed analyses have mostly dealt with certain cortically initiated 'excitatory' effects where an increase in sympathetic discharge has been demonstrated Little attention appears to have been paid to cortical structures that exert an inhibitory influence on the sympathetic control of the circulation Therefore with regard to the organization and functional significance of cortically induced depressor responses little is at present known despite the fact that cortical structures inducing a tonic inhibitory influence on lower sympathetic centres may well be functionally as important as those from which an enhanced sympathetic discharge can be elicited The behavioural changes with extensive sympathetic outbursts seen in the decorticate animal may, for instance suggest that lower sympathetic centres are normally exposed to some extent to a cortical inhibitory influence Further it should be realized that a generalized increase of a cortical inhibitory influence on the sympathetic control of the cardiovascular system may very well produce changes as dramatic as those elicited when excitatory cortical autonomic structures are excited Lastly it is not impossible that an inhibitory cortical influence can produce also fairly selective inhibitions of constrictor fibre tone affecting some vascular beds only If so fairly marked blood flow redistributions can be expected to occur with only small or moderate shifts in arterial blood pressure

Thus a more detailed analysis of cortical structures exerting an inhibitory influence on tonic sympathetic activity may reveal mechanisms of fundamental importance for cardiovascular control in the intact organism For several reasons the autonomic structures within the limbic system are here of particular interest Earlier exploratory experiments have indicated the existence of extensive limbic structures that appear to exert powerful inhibitory influence on cardiovascular function In addition data are accumulating which suggest that the limbic system may be of particular importance in connection with emotions and emotional expressions of which autonomic reaction patterns are known to form an important part

The limbic system or — as it is sometimes called — the visceral brain (MacLean 1949) was earlier thought to subserve essentially olfactory functions but research of the last decades has revealed that its function is much more complex than was earlier realized Papez (1937) introduced the hypothesis that the limbic system should be essential for the creation of emotions as well as for the elicitation of emotional expression Almost simultaneously

KLÜVER and BUCY (1937, 1938, 1939) demonstrated that in the monkey, lesions in different cortical regions within the limbic system drastically altered emotional behaviour

The theoretical speculations of PAPEZ and the striking experimental results obtained by KLÜVER and BUCY focussed neurophysiological and psychological interest on this phylogenetically old part of the telencephalon, and on the basis of these works a great number of studies has been performed (for reviews see BRADY 1960 and KAADA 1960) There has, so far, been no experimental evidence indicating any strict topographical organization for the different emotional components but there are numerous experimental and clinical observations proving that various kinds of emotional behaviour are closely linked to the limbic system as a whole According to KAADA (1960) in his critical review of the pertinent literature, the limbic system should not be regarded simply as a 'centre' of emotional behaviour and expressions but as the organic substrate of a number of more or less separate functions It should thus rather be looked upon as being constituted of several more or less well differentiated sections with different projections, where only parts of the functions are known at present It seems reasonable to assume that along with increasing encephalization, the limbic system has been developed to a different extent with various stages of complexity, and that it may be connected with, or devoted to, somewhat different functions in various species However it has been demonstrated that there exists *in primates* and *many other higher mammals*, including the cat and the dog, an extensive autonomic representation within this system, although the area for 'the visceral brain seems to be restricted to the anterior cingulate gyrus to the orbito-insulotemporal polar region and to the amygdala (KAADA 1960, p 1368)

From the studies by MCCULLOCH (1944) and SMITH (1945) it *is clear that* the rostral part of the cingulate gyrus *is one of the* most powerful suppressor regions and that within this area *there exists* an extensive autonomic representation *from* which effects on the cardiovascular system can be induced

The effect of *ablation* of this region has previously been studied in monkeys (WARD *1948* BARD 1950 GLEES *et al* 1950 PRIBRAM and FULTON *1954*, MIRSKY ROSVALD and PRIBRAM 1957), *in cats (*ROTHFIELD and HARMAN 1954 KENNARD 1955) and in man (for ref see KAADA 1960, p 1366) In monkeys such ablations usually lead to changes in behaviour towards tameness Psychotic patients *seem to lose* their preoperative agitated or aggressive behaviour while corresponding *operations* in cats often tend to alter the emotional behaviour to a lesser degree or even in the *opposite* direction It is however difficult to know if the brain areas *extirpated* in the different species are analogous

Topical stimulations within these structures have yielded a multiplicity of responses and both excitatory and inhibitory somato and *viceromotor reactions* have been described Not seldom both *types of response can be obtained* from one and the same point of stimulation depending on *e g* the *stimulation characteristics and the type and depth of anesthesia* This probably *means that within these areas there exists* a structural overlapping of neuron pools and fibres mediating functionally different patterns as will be discussed further in subsequent chapters As far as the somatomotor *system is concerned* an inhibition of muscular tone and respiration as well as suppression *of cortically and reflexly induced movements* has generally been obtained from the areas immediately surrounding the genu of the corpus callosum (HODES PEACOCK and HEATH 1951 KAADA 1951) In other areas facilitation of skeletal muscle activity has been obtained but here also the relation between function and structure appears to be complex with a considerable *spatial overlap* (KAADA 1960)

With regard to the effects observed in *conscious* animals the most easily evoked response from topical stimulations of the anterior cingulate and subcallosal gyrus seems to be an arrest or attention reaction This special type of response in the conscious animal appears mostly to be combined with excitatory effects on the visceromotor system with pupillary dilatation blood pressure rise etc (KAADA 1960 p 1362) It is also of interest to note that these arousal effects are sometimes *obtained from areas from which in the* anesthetized animal an inhibitory effect on somatomotor activity is obtained Whether a generalized inhibitory response affecting both somatomotor and visceromotor control can be obtained in the unanesthetized animal is not quite clear so far possibly the neuron pools are spatially so intermingled that in most areas *stimulated the excitatory* response patterns will dominate the animal's behaviour It is thus difficult to judge from available results of topical stimulations how visceromotor and somatomotor reactions are normally combined to form special behavioural patterns

With special reference to the cardiovascular changes obtained by topical stimulations within the rostral cingulate gyrus there are several studies where effects on arterial blood pressure and or heart rate have been observed *In monkeys lightly anesthetized with ether* SMITH (1945 1949) observed that electrical stimulation within the rostral third of the cingulate gyrus led to either an increase of the arterial blood pressure without any concomitant alteration of the heart rate or to a blood pressure fall associated with a more or less marked bradycardia The negative chronotropic heart effect which was said to be abolished after vagotomy was in some experiments very marked and even complete cardiac arrest was noted in a few experiments

on conscious animals leading to a dramatic fall in systemic blood pressure Since vagotomy more or less completely eliminated the bradycardia and the blood pressure fall the stimulated structures apparently did not induce any significant depression of the tonic sympathetic activity Vagal slowing and even temporary arrest of the heart was also later observed by WARD (1948) in Dial anesthetized monkeys as a response to electrical stimulation within the same cortical region These effects sometimes occurred without any concomitant effects on *e g* respiration and pupils In dogs anesthetized with Dial KREMER (1947) noted small but definite depressor effects when stimulating the supracallosal anterior part of the cingulate gyrus In an extensive study of rhinencephalic and other structures in monkeys dogs and cats under various types of anesthesia KAADA (1951) usually observed a blood pressure decrease as a response to electrical stimulation within the pre and subcallosal region and other cortical areas in close anatomical relation to the limbic system However blood pressure elevations were also noted both in this study and in a previous one (KAADA PRIBRAM and EPSTEIN 1949) Bilateral section of the fornix had no effect on the blood pressure changes elicited The induced depressor effects which were more marked in the monkey than in the cat were usually of the order of 15—30 mm Hg Bilateral vagotomy generally reduced the responses markedly but did not abolish them completely a simultaneous inhibition of tonic sympathetic activity may thus have been involved The possibility that the sympathetic dilator fibres might also have been activated cannot however *a priori* be excluded In explorations of the diencephalon and adjacent parts of the cerebral cortex HESS and co workers also performed topical stimulations within the cingulate gyrus (AKERT HESS and McDONALD 1951 HESS AKERT and McDONALD 1951) They reported that stimulations of this part of the cerebral cortex mostly led to a blood pressure increase in cats anesthetized with Dial Similar effects have been reported by ANAND and DUA (1956) who noted that the blood pressure rises had no relation to the concomitantly produced changes in heart rate In their study of the autonomic representation within the cerebral cortex WALL and DAVIS (1951) performed 2 *experiments* where rostral parts of the cingulate gyrus were topically stimulated in monkeys From this region blood pressure changes of the order of 10—20 mm Hg were obtained They suggested that these effects were dependent on intact temporal lobes and that the centrifugal fibres apparently did not pass via the hypothalamus

In the studies quoted above where cardiovascular changes elicited from the anterior cingulate gyrus and the subcallosal gyrus were obtained no attempts were made to evaluate critically the cortically induced depressor effects with

Topical stimulations within these structures have yielded a multiplicity of responses and both excitatory and inhibitory somato and visceromotor reactions have been described Not seldom both types of response can be obtained from one and the same point of stimulation depending on e g the stimulation characteristics and the type and depth of anesthesia This probably means that within these areas there exists a structural overlapping of neuron pools and fibres mediating functionally different patterns as will be discussed further in subsequent chapters As far as the somatomotor system is concerned an inhibition of muscular tone and respiration as well as suppression of cortically and reflexly induced movements has generally been obtained from the areas immediately surrounding the genu of the corpus callosum (HODES PEACOCK and HEATH 1951 KAADA 1951) In other areas facilitation of skeletal muscle activity has been obtained but here also the relation between function and structure appears to be complex with a considerable spatial overlap (KAADA 1960)

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give a more general survey of the types of cardiovascular effects that can be induced from these cortical structures (see Chapter III) Further, a comparison has been performed between the cortically induced depressor responses and depressor effects that can be elicited from lower levels of the central nervous system and from some of the cardiovascular receptors To facilitate such a comparison it proved necessary to analyse also some aspects of the tonic sympathetic discharge of the medullary vasomotor centre and its reflex control The results of this latter part of the study, earlier described elsewhere (Folkow Johansson and Lofving 1961, Lofving 1961) will be briefly mentioned in this paper (Chapter IV) as they are important for the evaluation of the cortically induced sympatho inhibitory responses The preliminary results of the present study have previously been briefly reported (Lofving 1960 Folkow 1961)

special regard to their influence on the tonically active sympathetic fibres of the heart and the blood vessels Even where it has been observed that blood pressure decreases can still be elicited after vagotomy it is not definitely known whether these relatively small falls in blood pressure were caused by an inhibition of a prevailing discharge in the vasoconstrictor fibres or in the cardioaccelerator fibres or in both of these Further as earlier mentioned dramatic regional vascular adjustments may occur without being much reflected in the systemic blood pressure An activation of the sympathetic vasodilator fibres might even have contributed to the pressure fall and there is also the possibility that at least in some cases centrally induced changes of skeletal muscle activity or respiratory changes may have so affected the circulation as to lower the blood pressure In summary therefore although much evidence exists to indicate a considerable autonomic representation within the cingulate gyrus affecting the circulation the peripheral patterns of cardiovascular responses which may be elicited therefrom are almost entirely unknown This is somewhat surprising having regard to the fact that this area is probably closely concerned with emotional feelings and expressions and hence likely to be intimately associated with those cardiovascular changes which are so often observed as manifestations of emotional reactions

During recent years investigations have been performed in this department in order to study more closely problems concerned with the discharge patterns of the adrenergic cardiovascular control system In order to gain a better quantitative insight into this field attempts have been made to explore (1) the range of control of the sympathetic fibres over different types of cardiovascular effectors (2) the physiological discharge rate of the sympathetic vasomotor fibres and (3) the cardiovascular effects exercised by the direct innervation as compared with that of the hormonal component of the sympatho adrenal system (for ref see FOLKOW 1960)

These studies have made it easier to analyse in more detail the sympathetic patterns induced when the medullary vasomotor centre is exposed to various excitatory and inhibitory influences emanating from receptors and from higher autonomic centres (see e g FOLKOW JOHANSSON and ÖBERG 1959 FOLKOW et al 1960)

In the present study an attempt has been made to analyse in more detail the nature and the extent of the cardiovascular depressor response pattern that can be elicited from the anterior parts of the limbic system notably the cingulate and the subcallosal gyrus In the course of this investigation pressor effects were repeatedly observed from structures closely adjacent to those from which depressor reactions could be elicited Some aspects of these pressor response patterns will subsequently be briefly outlined in order to

then, for the present purpose, be forced to use preparations of fine bundles of postganglionic C fibres of known distribution. Then, however, the recording difficulties are very much increased with correspondingly increased difficulties of judging the extent of regional discharge changes. If recordings from single C fibre preparations were performed it would be necessary to study the single unit discharge not only from one region, but simultaneously from the vasoconstrictor fibre supply of several vascular beds to ascertain the discharge pattern induced. This would, however, be technically almost impossible, both with regard to preparing and keeping in good condition several isolated C fibres and to the exceedingly small action potentials of such fibres, which are close to the noise level of the amplifier. In addition, one could never be certain whether the discharge rate of the particular fibres being recorded really was representative of the *average* discharge rate of all the fibres running to the respective vascular beds. It is, after all, the average discharge activity of all the fibres distributed to a vascular region which determines the extent of the neurogenically induced effector response.

There are in addition more special drawbacks, unavoidable if *only* the action potentials are recorded. The action potentials of e. g. a sympathetic constrictor and a sympathetic dilator fibre are probably indistinguishable, although to the effector cells they mean a great difference. Accordingly, it would in all circumstances be essential to record also the effector responses in order to have a complete survey of the actual discharge pattern. *For these and other reasons certain aspects of the functional characteristics of the autonomic nervous system appear to be most suitably approached by the use of quantitative recordings of the effector responses, at least at present, rather than by studying fibre activity directly.*

The correlation between discharge rate and effector response forms an initially very steep, hyperbolic curve for most sympathetic neuroeffector units, so that even minor shifts in physiological discharge produce considerable shifts in effector response. Providing other factors can be kept fairly constant an approximate value for the average sympathetic discharge rate can therefore be deduced from the recorded effector responses. With respect to neurogenically induced shifts in flow resistance, such deductions are greatly facilitated by the fact that the shifts in effector lenght and thus in the internal vascular radius are reflected in the blood flow recordings as magnified to their fourth power (Poiseuille's law). Each vascular bed has a fairly characteristic curve relating constrictor fibre discharge to the ensuing shifts in flow resistance (CELANDER and FOLKOW 1953, CELANDER 1954). This fact makes it relatively easy, when experience is gained, to reveal even fairly small shifts in constrictor fibre discharge (see FOLKOW 1955, 1960).

CHAPTER II

Methods

1. Critical evaluation of the experimental approches

In the present study attempts have been made to estimate cortically induced alteration of regional vasoconstrictor fibre tone on the basis of effector responses calculated in terms of flow resistance changes This implies simultaneous recordings of arterial pressure and venous outflows from different vascular regions and involves rather extensive operative manipulations At the same time the excitability of the delicate cortical structures easily interfered with by even small amounts of anesthetics or slight disturbances in the general condition of the animal must be good to allow effective excitation of the neurons influencing the circulation

On the other hand a purely electrophysiological approach to the problem outlined in the present paper cannot give precise information about the details of the cardiovascular response patterns elicited It should be realized that gross recording from bigger fibre bundles technically relatively easy to perform is hardly able to reveal small or moderate regional differences in changes of discharge rate It should be recalled that even small regional differences in average discharge rate — so small that at first sight they would seem to be neglgible — can have profound effects on the blood flow in different tissues partly due to the frequency response characteristics of the particular vascular regions Suppose that the constrictor fibres of one vascular area are only slightly less activated than those of the others in a pressor response Haemodynamically such minor differences in discharge can have the result that the blood flow of this tissue in fact *increases* due to the simultaneously raised perfusion pressure while the flow in the other tissues *decreases* somewhat due to the fact that their regional resistance increases relatively more than the perfusion pressure

Further if action potentials were recorded from isolated preganglionic fibres as is usually the case in detailed electrophysiological studies of the autonomic nervous system it would be very difficult to know to which particular vascular section the fibres under study were distributed One would

ting to 35 cats, are not included in this study since they cannot reasonably be regarded as representative However, it was never observed that under such circumstances the responses to cortical stimulation changed qualitatively, for instance a depressor response never altered to become a pressor one with one and the same type of topical stimulation of a given cortical structure

When some experience was gained the total time for preparation could be reduced to about 2 hours Care was always taken to keep the body temperature of the animals constant at 38° C Bleeding was carefully avoided, and if it accidently occurred the estimated volume was substituted with dextrane Tyrode solution

b Operative procedures on the head and neck A tracheal cannula was inserted to allow free passage of air during the preparation and also to allow for artificial respiration in the experiments when the animals were curarized, or where it was essential to avoid respiratory changes during the experimental procedures The vagi the aortic depressor nerves and the common carotid arteries were carefully dissected free in the neck to facilitate elimination of the aortic and carotid baro and chemoreceptors in the course of the experiment This was later performed by cutting the dissected nerves bilaterally and in most cases, by clamping the carotid arteries As, however, in some experiments clamping the common carotids appeared to interfere with the blood supply of the stimulated brain structures, the carotid sinus regions were in such animals infiltrated with a 2 per cent Xylocaine solution in amounts seldom exceeding 1 cc so as to block the chemo and the baroreceptor fibres within the sinus nerves

After a longitudinal incision in the skin above the calvarium and removal of the temporal muscles from their origin, the skull was opened by means of a dental drill over an area of about 1 square cm bilaterally to the sagittal suture just above the cingulate gyrus and anterior parts of the hypothalamus Great care was taken not to interfere with the venous drainage of the brain via the superior sagittal sinus Bleeding from the bone, which normally occurred during this part of the preparation, was immediately stopped by plasticine applied to the cut bone surface Cooling and drying of the brain was avoided by leaving the dura intact until the electrodes were inserted, and the skull was again covered by suturing the skin The head of the cat was then mounted in a stereotaxic instrument — Horsley Clarke apparatus (see below) — allowing for topical application of stimulation electrodes in any desired part of the forebrain When stimulation of the brain structures was to be started, the skin was again opened the dura cautiously punctured to allow entrance

2 Experimental procedures

a Material and anesthesia On a total of 103 cats technically successful experiments were performed on 68 animals with bodyweights varying between 2 4—4 5 kg the mean being 3 2 kg After preliminary induction with ether anesthesia was adjusted to a superficial plane by the intravenous injection of small doses of chloralose carefully avoiding any excess When this was satisfactorily established the animals exhibited brisk somato and visceromotor reflexes The doses required for such a superficial anesthesia varied somewhat from one animal to another but usually 30—40 mg/kg bodyweight had to be given

In a minor series of 9 experiments performed early in the present study other anesthetics were used Urethane up to 1 5 g/kg was used in 3 animals a mixture of chloralose and urethane in 2 animals nembutal in a dosage of about 20—25 mg/kg in 2 animals and lastly in 2 animals ether was given throughout the experiment in a closed circuit arrangement If great care was taken to keep the animals only superficially anesthetized these anesthetics did not alter the main characteristics of the effects induced under chloralose As however chloralose seemed to be best suited for maintaining a reasonable excitability of the cortical autonomic centres this substance was used in most of the experiments

In this light anesthesia it was found to be of the utmost importance to reduce trauma to the animals to a minimum otherwise they were very easily brought into a shocklike condition and then responded poorly to induced cardiovascular reflexes Consequently during those operative phases which could be expected to be traumatic the depth of anesthesia was temporarily increased by administration of gaseous anesthetics For this purpose an anesthetic apparatus (AGA Anestor universal UDNK 23) was used adjusted to give nitrous oxide (N_2O) and oxygen in proportions 80 20 If required small amounts of ether could be added to the gas mixture In this way the depth of anesthesia could be rapidly adjusted to any desired level

By careful control of the animals with regard to anesthesia avoidance of bleeding and trauma it was possible to perform technically successful preparations in a comparatively high proportion of experiments (66 per cent) In these experiments quantitatively fairly good responses were obtained on topical stimulation of cortical autonomic structures However whenever the animals deteriorated in some way or other the autonomic reactions obtained on *e g* cortical stimulation generally became less and less pronounced This was a common feature at the beginning of the present investigation before sufficient experience and training had been gained Such experiments amoun

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of the electrodes and the surface of the *brain covered with liquid paraffin* or saline at body temperature

In later experiments of the present series the skull was opened in another and less traumatic way For this purpose a special drill instrument was made In an ordinary Horsley Clarke carrier a metal rod was mounted in a bearing A steel drill of 0 6—1 2 mm diameter was inserted into a chuck fitted to the bottom of this rod which could be brought into rotation by a dental drill connected to its top via a flexible transmission When the head of the animal *had been placed in position in the stereotaxic instrument* the skull was exposed and during rotation the drill was slowly moved along the selected ordinate so as to penetrate the bone It proved possible to drill through the skull above the actual cortical region with a high degree of accuracy The dura was then cautiously punctured and the electrodes introduced into the brain via the drilled channels This operative procedure offered great advantages since it reduced the time as well as the extent of the preparation but a *post mortem* examination with careful control of the stimulation points was of course required

In those experiments where it was found desirable to obtain access to the medulla oblongata the occipital part of the skull and the dorsal part of the first cervical vertebra were exposed The lower margin of the occipital bone was then drilled out in the midline over an area 3 mm broad and 5 mm long In some experiments the caudal part of the cerebellum was removed by cautious suction so as to expose the caudal end of the floor of the fourth ventricle in other experiments the localization of the medullary depressor area (see Chapter V) could be secured without damaging the cerebellum significantly A concentric electrode could then be orientated in the medullary depressor point for topical stimulations or for the production of localized electrolytic lesions

c Operative procedures for blood flow recordings Blood flow in *skeletal muscles* was recorded in the calf of one of the limbs As it proved very important to reduce the time of preparation as well as the traumatization in the lightly anesthetized animals the hind limb was usually *not skinned However* in order to decrease the contribution from cutaneous blood flow the circulation through the paw was excluded by a tight ligature around the ankle and the draining cannula for blood flow recording was inserted into the deep portion of the femoral vein distally to the entrance of the saphenous vein In five experiments a completely isolated skeletal muscle preparation was used in which the skin was entirely removed and the circulation of the paw excluded The cognate vessels and the nerves of the calf were then cautiously dissected

free at the level of the knee joint where the popliteal vein draining the calf muscles was cannulated for recording the blood flow The venous outflow was then led to a closed drop chamber filled with silicone oil after which it was returned to the animal via a suitable vein The apparatus used for the continuous recording of the blood flow is described below (e) To avoid drying and cooling the calf was wrapped in a plastic cover moistened with saline and warmed by a heating lamp to a temperature of about 35° C

One of the hind paws constituted the vascular region chosen for measurement of the *skin* blood flow The greater saphenous vein was prepared free at the ankle joint and cannulated Other superficial veins at this level were ligated great care being taken to avoid damage to superficial nerves carrying vasomotor fibres to the skin vessels The flow was recorded by a drop recorder ordinate writer unit as described below (e)

In order to prepare the *kidney* for measuring its blood flow a dorsal retroperitoneal approach was found to be suitable as the superficially anesthetized animals proved very sensitive to intra abdominal manipulations The kidney pedicle was then exposed through a slit between the paravertebral muscles of the back and under magnification the renal vessels were freed for a distance of 5 7 mm while great care was taken to avoid damage to the renal nerve plexus During the cannulation of the vein of the kidney the renal artery was briefly occluded by a gently applied rubber covered bulldog clamp The venous outflow was led to a drop chamber as described below (e) and the outflow from the drop chamber was returned to the animal via the proximal end of the renal vein or if this proved impossible to the external jugular vein Whenever renal blood flow was measured the intactness of the sympathetic innervation of the kidney was always tested at the end of the experiment by inducing a neurogenic renal vasoconstriction usually by a hypothalamic stimulation sometimes by asphyxia Only those experiments were used for evaluation of the cortically induced neurogenic patterns where such tests proved that the innervation of the renal vessels was intact

To record centrally induced neurogenic changes of *intestinal* blood flow a part of the proximal jejunum was tied off leaving its nervous and vascular supply intact The rest of the intestines was removed The superior mesenteric vein draining the jejunal loop was then cautiously isolated and cannulated for measuring the venous outflow from the region as described below (e) The blood was returned to the animal via the central end of the same vein

d Recording of blood pressure and blood flow Arterial blood pressure was continuously recorded throughout the experiments After administration of heparin (5 mg kg bodyweight) the femoral or brachial artery was cannulated

and connected to a mercury manometer via a polyethylene tube filled with saline Heart rate was usually estimated by counting the pulse rate but exceptionally recorded by an electrical pulse rate counter operating an ordinate writer

To allow a closer analysis of the centrally induced cardiovascular reaction patterns regional blood flows were recorded throughout the experiment Since in most experiments it proved technically impossible to record simultaneously more than two blood flows together with maintained good cortical excitability it was necessary to use one of them as a sort of reference to allow comparison between the quantitative effect in different experiments For this purpose skeletal muscle blood flow was found most suitable because the vascular reactions elicited within this region seemed to be most constant and closely related to the level of the initial tonic activity of the vasoconstrictor fibres

Venous outflow from the skeletal muscle region was generally recorded and concomitantly with the blood flow from one of the kidneys in a total of 18 experiments with that from a selected part of the jejuno ileum in 6 experiments and with that from the skin of the paw where the pad circulation had in most experiments been excluded by placing clamps or ligatures around the pads in 20 experiments In 10 successful experiments blood flows were recorded in pairs from analogous tissue regions where the vessels on one side had been acutely sympathectomized with respect to intestinal blood flow recordings were made before and after cutting the sympathetic supply to the vessels The flow changes in the denervated vascular bed were then used as a control for judging the extent of the neurogenically induced flow changes in the corresponding tissue with an intact vasomotor innervation In this way blood flows were recorded from skeletal muscle regions in 3 from skin regions in 2 from the kidneys in 3 and lastly from the intestines in 2 experiments

The regional venous outflow was continuously measured throughout most of the experiments by means of two or more recording units each made up of a drop chamber and an optical drop counter unit operating an ordinate writer (CLEMENTZ and RYBERG 1949) These flowmeters are so designed that the heights of the ordinates are approximately *inversely* proportional to the rate of flow The venous outflow from the respective regions was directed from the cannulated vein to the closed drop chamber via a polythene tube and then returned to the animal through a suitable vein usually the proximal end of the same vein The flow resistance in these extracorporeal flow circuits was minimized by using tube connections as wide and short as possible Each drop chamber together with the connecting tubes had a volume of 3–4 cc

To start with they were filled with dextrane Tyrode solution and silicone oil so that the blood drops fell through an inert low density fluid medium (LINDGREN 1958)

e Elimination of the adrenal medullary secretion Initially or during the course of the experiments the secretion of the adrenal medullae was eliminated in 12 animals This was done by ligating the vessels of the right and sectioning the sympathetic nerves to the left adrenal gland This preparation ensured the elimination of reflexly released adrenal catechol amines without interfering with the secretion of the cortical hormones from the left adrenal However to avoid the traumatic effect of a laparotomy or the time consuming operation of a retroperitoneal approach to the suprarenals the glands were left intact in most experiments This was considered justified since in preliminary experiments it was repeatedly observed that the effects of cortically induced changes in blood pressure and regional blood flows were not significantly influenced by a concomitant reflex release of catechols from the adrenal medullae It is also known that the hormonal component is with few exceptions of relatively insignificant importance concerning excitatory effects on the vessels as compared with their direct innervation (CELANDER 1954)

f Artificial respiration After initial observations of cortically induced cardiovascular changes the animals were curarized by administration of decamethonium slowly injected intravenously in doses of 0 5 mg/kg bodyweight The respiration was then maintained by a Starling Ideal pump which was so adjusted before the injection that the frequency of the respiration corresponded to the rate of the spontaneous respiration and the tidal volume delivered barely suppressed the spontaneous respiration and left arterial blood pressure unchanged When required additional injections of decamethonium were given later during the experiments Curarization was performed to avoid changes in blood pressure and blood flows secondary to changes of skeletal muscle activity which otherwise occurred spontaneously or as a response to electrical stimulation of the different nervous structures

g The stereotaxic instrument For topical electrical stimulation of the brain structures the stereotaxic technique of Horsley Clarke was utilized The instrument used consisted of a metal frame carrying two or more electrode holders which were independently movable in three planes at right angles to each other The head of the animal was fixed in such a way that the external auditory meatuses and the inferior orbital ridges coincided with the basal

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about 0 2—0 4 mA The magnitude of the response was, of course, dependent on the duration of each stimulus if this was brief Generally the effect was about maximal at 3—5 msec at the voltage used, and hence this range of pulse duration was usually applied The stimulation frequency was varied within wide limits from about 2 impulses sec to more than 100 impulses/sec

When an *electrolytic lesion* in the sympatho inhibitory hypothalamic area was to be accomplished, two pairs of concentric electrodes were directed towards the points giving a maximal depressor response at low voltage sti mulation at frequencies around 50/sec When the optimal position was reached the positive pole of a 120 V battery was connected to the central core of the electrode, and the negative pole to an indifferent electrode, attached to the animal through a slit in the skin A d c of about 10 mA applied for 1—3 min produced a strictly localized electrolytic lesion (JASPER and AJMONE MARSAN 1960)

The stimulation characteristics used for topical excitations of the 'depressor area within the bulbar cardiovascular centre were usually 0 5 V, 3 msec and 50—90 impulses/sec Electrolytic lesions within this area were produced in the same way as described above

c *Implantation of electrodes in 'chronic animals'* In an additional series, performed on 5 cats the effects of stimulations of the rostral cingulate gyrus on general behaviour were observed in conscious animals Under nembutal anesthesia and with aseptic precautions the skull was prepared as above and fixed in the Horsley Clarke apparatus Two pairs of platinum iridium elec trodes diameter 0 3 mm, insulated as above, were directed bilaterally towards that area in the rostral cingulate gyrus from which maximal sympatho inhibitory effects were known to be elicited in the anesthetized animal When the electrodes were correctly positioned the perspex plate in which they were fixed was sealed to the skull with acrylic resin, the latter covered both the plate and three stainless steel screws which were inserted into the bone to facilitate fixation Insulated silver leads connected to the electrodes were passed under the skin of the head and neck down to the level of the second thoracic vertebra and via a slit in the skin brought out to a connector, attached to a chamois leather jacket (ABRAHAM, HILTON and ZBROZYNA 1960) Stimulation of the conscious animals with chronically implanted electrodes was carried out with 1—3 V 2 5 msec and 20—80 impulses/sec

plane of the instrument which in this investigation was made the zero horizontal plane

The coordinates for defining the position of the cortical negative electrode are throughout this paper given according to mathematical conventions and refer to a standard cat of 3 0 kg The intersection between the median and the zero horizontal planes forms the abscissa in a coordinate system and perpendicularly to this line in the median plane the ordinate is drawn The third coordinate axis is coincident with the interaural line whose intersection with the median plane is taken as the origin As the negative electrode is placed just within the cortical tissues and hence the distance from the median plane is constant (about 2 mm) throughout the experiments this coordinate is not indicated in the figures

If cats of approximately equal size are used this stereotaxic method allows a most accurate localization of the brain structures and the tip of an electrode can be placed at any desired point within ± 0 5 mm (JASPER and AJMONE MARSAN 1960)

b Electrodes and type of stimulation For cortical stimulation bipolar electrodes were used each made up of steel wires with a diameter of 0 3—0 8 mm insulated with enamel except at the tip Stimulation was always performed bilaterally with bipolar electrodes which could be placed in two principally different positions so that the electrical field was essentially directed either *in the sagittal or in the frontal plane on both sides The electrodes placed* caudally respectively medially were always used as the negative poles The distance between the positive and the negative electrodes in each pair varied It was usually 2 mm and never exceeded 3 mm The distance between the two negative electrodes placed on each side of the sagittal suture was *3—4 mm so that the electrodes were strictly* located within the cortical tissue this was checked after every experiment

For *hypothalamic* and *medullary stimulation* as well as for inducing electrolytic lesions concentric bipolar electrodes were used each consisting of an insulated nichrome cylinder surrounding an insulated central wire Under magnification the insulation at the tip was carefully removed

Electrical stimulation was performed with a Grass stimulator delivering square wave stimuli and designed to permit variation of the voltage duration and frequency independently *During the course of this study this instrument* was from time to time tested with a cathode ray oscilloscope so as to ensure correct performance The stimulation voltage used for cortical stimulations was comparatively low and clear cut responses were mostly obtained with 2—4 V The tissue resistance was periodically tested and usually found to be *10 000—15 000 ohm which means that the tissue was exposed to a current*

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When an *electrolytic lesion* in the sympatho inhibitory hypothalamic area was to be accomplished two pairs of concentric electrodes were directed towards the points giving a maximal depressor response at low voltage stimulation at frequencies around 50 sec When the optimal position was reached the positive pole of a 120 V battery was connected to the central core of the electrode and the negative pole to an indifferent electrode attached to the animal through a slit in the skin A d c of about 10 mA applied for 1—3 min produced a strictly localized electrolytic lesion (JASPER and AJMONE MARSAN 1960)

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CHAPTER III

Some principal features of limbic autonomic areas affecting the nervous control of the cardiovascular system

The findings to be outlined in this chapter were to a great extent obtained in an early exploratory series of experiments. They are described here mainly in order to present a general orientation of the complex and sometimes highly differentiated cardiovascular adjustments that can be elicited from rostral parts of the limbic system. *It was found that both 'excitatory' and 'inhibitory' influences on the tonic sympathetic cardiovascular control could be elicited.* The excitatory effects sometimes affected the blood flow distribution in such a way as to suggest a differentiated cortical influence on the sympathetic neuron pools controlling the different vascular circuits. It was evident that even the cortical *inhibitory effects, the analysis of which is the primary aim of the present investigation,* did not diminish the flow resistance of the various vascular beds to an equal extent, a fact that will be dealt with in detail in Chapter IV.

Early in this study it was realized that the organization of these cortical visceromotor neurons was very complex. To be fully understood it required extensive analysis and needed to be correlated with other aspects of the function of the limbic system. Accordingly it proved necessary to limit the scope of the present investigation and to exclude from the further more detailed experimental analysis the accidentally observed and often very complex excitatory responses, *interesting though they may be.* They will be more fully dealt with in subsequent studies, but it will be convenient to present here only some scattered data which will serve to illustrate some general characteristics of the limbic cardiovascular centres and act as an introduction to the subsequent chapters.

Results. Systematic explorations of the cingulate gyrus soon confirmed earlier investigations (see Chapter I) that the areas affecting cardiovascular nervous activity are concentrated rostrally. From the caudal two thirds no cardiovascular responses whatsoever could be elicited by topical electrical stimulations except when very high voltage was used. However, from the rostral third, especially from the area around the genu of the corpus callosum, blood pressure falls were easily obtained, while more ventrally within the

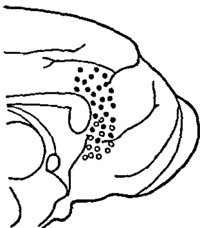


Fig 1 Diagram of the anterior medial surface of the cat brain showing approximate localization of points yielding depressor (closed circles) and pressor (open circles) effects upon electrical stimulation Note the considerable overlap of the two areas

subcallosal region topical stimulations usually elicited rises in systemic blood pressure Still more ventrally within the medial cortex, situated rostrally to the hypothalamus topical stimulations did not generally cause any very significant blood pressure changes, but proved to affect cutaneous blood flow markedly

Fig 1 illustrates the approximate location of the above mentioned areas which exert inhibitory and excitatory effects on tonic sympathetic control of the circulation To simplify their description they will subsequently be referred to as the depressor' and 'pressor areas The delineations of these areas are based upon several hundred topical stimulations where systemic blood pressure and generally one, or several regional blood flow recordings were performed in experiments so devised as to exclude possible artefacts (see Chapter II) It is seen from the figure that a considerable overlap exists between the depressor and pressor areas In the lateral direction they extend about 5–6 mm from the midline, but the cardiovascular effects are most easily elicited from the medial sections

The cardiovascular responses which could be elicited from the limbic depressor and pressor areas studied, were usually small in extent (at best 20—30 mm Hg) when the powerful baroreceptor mechanism operating via the bul bar cardiovascular centres was left entirely intact This finding was not unexpected for several reasons which are discussed in more detail in the last part of this chapter It ought, however, to be mentioned here that even in lightly anesthetized animals the excitability of cortical structures is severely depressed whilst reflex adjustments relayed at bulbar levels can be expected to be only little interfered with Consequently, it was considered essential to allow the cortically induced neurogenic patterns to exert their influence with a minimum of damping or counteracting adjustment For this reason most of the present results were obtained in animals where — except for the

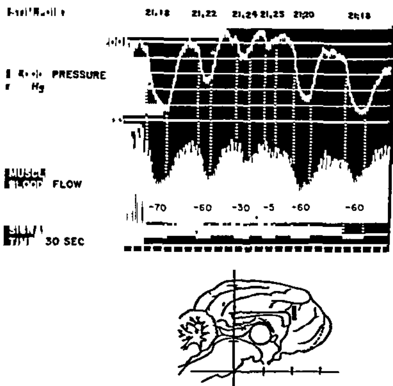


Fig 2 Cat 4 2 kg Chloralose Effects on arterial blood pressure and skeletal muscle blood flow of bilateral bipolar electrical stimulation (3 V, 3 msec, 20 imp /sec) at different points within the depressor area of the rostral cingulate gyrus Note that slight shifts in electrode position change the responses quantitatively but not qualitatively

In the experiments illustrated *in this and the following figures the animals were* atropinized curarized and kept under artificial respiration The vagi and the aortic depressor nerves were cut in the neck and the common carotid arteries bilaterally occluded — The numbers superimposed on the blood flow recordings represent approximate values for peripheral resistance changes expressed as per cent of control — *The heights of the ordinates are inversely proportional to the rate of flow*

initial experimental period — the cardiac, aortic and carotid cardiovascular receptors were either denervated or kept at a fairly steady activity level during the cortical stimulations Such a procedure was found to be justified since elimination of the influences of the cardiovascular receptors was observed to affect only the extent but not the essential nature of the cortically induced cardiovascular adjustments Thus, the depressing *effect of the anesthesia* on the cortical structures was experimentally partly compensated for by the elimination of a normal damping mechanism

The experiments illustrated in the subsequent figures are *only* representative

for the effects generally obtained on topical stimulations within the rostral cingulate and subcallosal regions when the damping effects of the cardiovascular proprioceptors had been largely eliminated

Fig 2 shows a typical record from an experiment where bilateral bipolar stimulations are performed within the cortical tissue of the cingulate depressor area while the electrode position is slightly shifted between the stimulations The effects on arterial blood pressure and skeletal muscle blood flow are recorded under conditions where the sympathetic discharge had initially been increased by releasing the medullary vasomotor centre (VMC) from the damping effect of the baroreceptors This was performed by means of a temporary occlusion of the common carotid arteries when the vagal nerves and the aortic depressor fibres had been cut in the neck It is seen from the figure that the most pronounced depressor effect is induced from the first stimulated point According to the coordinate system employed this point (21 18) is situated 21 mm rostrally and 18 mm dorsally to the interaural line A blood pressure fall of 80 mm Hg is obtained, while at the same time muscle blood flow is about doubled in spite of the marked fall in perfusion pressure This means a decrease of regional flow resistance to less than one third of the initial value Slight shifts of electrode positions in the ventro dorsal direction change the extent of the depressor effects elicited despite the fact that the stimulation characteristics are the same In this experiment the vagal nerves had initially been cut and the reflex release of adrenal medullary hormones eliminated The animal was atropinized, curarized and in addition kept under constant artificial respiration The cardiovascular effects must then be ascribed to an inhibition of the tonic discharge of the sympathetic adrenergic fibres supplying the vessels and the heart The observations represented in Fig 2 suggest that the sympatho inhibitory effects may be relatively more pronounced in the skeletal muscle vessels than in those of most other regions a finding which will be further analysed in subsequent chapters

In the experiment illustrated in Fig 3 the electrodes are placed within the pressor area of the subcallosal regions where three consecutive electrical stimulations have been performed at point (21, 15) As usual when the animals were in good condition more or less marked blood pressure rises are obtained from this more ventrally situated area It can be seen from A and from B in this figure that the pressor response is combined with a pronounced blood flow decrease within the skeletal muscles a more moderate one within the intestines while the blood flow in the paw, dominated by the circulation through the arteriovenous shunts of the pads in fact increases somewhat In C of Fig 3 the arterial blood pressure to the regions under study

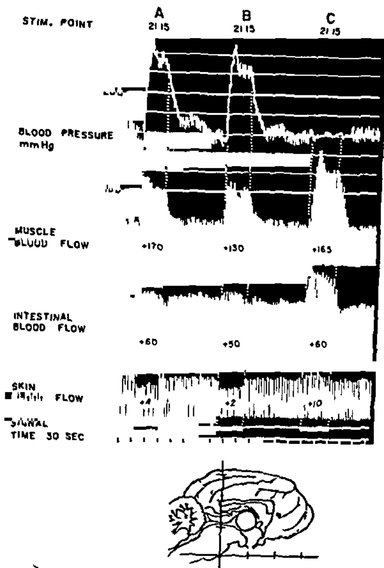


Fig 3 Cat 2 8 kg Chloralose Effects on arterial blood pressure and on blood f in skeletal muscle inte tinal and skin regions of bilateral bipol r electrical stimula (4 V 3 msec 60 imp /sec) at point (21 15) within the pressor area of the subcall region (A and B) In C the perfusion pressure to the regions under study is kept cons during the stimulation by *partial occlusion of the abdominal aorta* — For fur details see Fig 2

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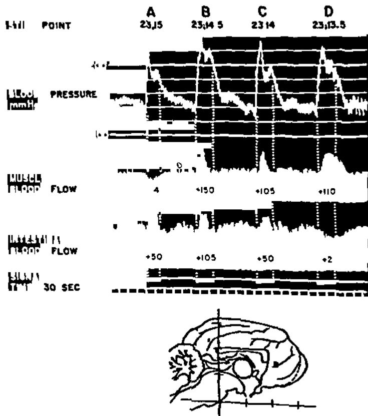


Fig 4 Cat 3 2 kg Chloralose Effects on arterial blood pressure, skeletal muscle and intestinal blood flows of bilateral bipolar electrical stimulation (3 V, 3 msec, 60 imp /sec) at different points (23 15—13 5) within the pressor area of the subcallosal region Note that in A the vasoconstriction is marked within the intestinal region and absent in the skeletal muscle while the opposite effect is obtained from a point 1 5 mm more ventrally, D
For further details see Fig 2

rogenic vasoconstriction in all three regions although the vasoconstriction in the skeletal muscles is by far the most prominent while that of the cutaneous vessels is very slight This order of effectiveness of the cortical pressor area with respect to the induced vasoconstrictions within the different tissues was usually obtained though occasionally, as illustrated below, a quite different response pattern has been observed The prompt appearance and elimination of the regional vascular responses makes it clear that they must be mediated

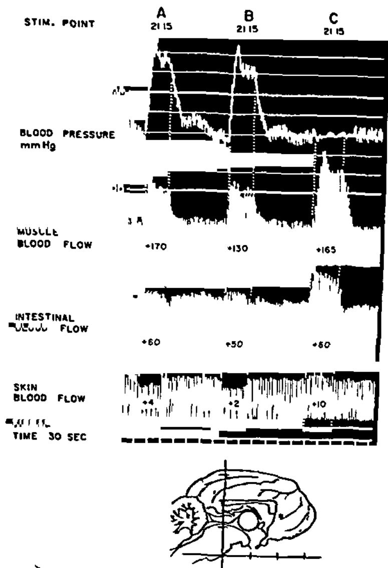


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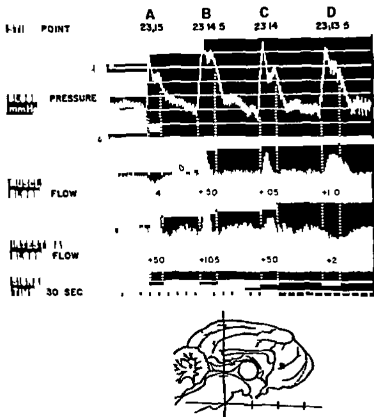


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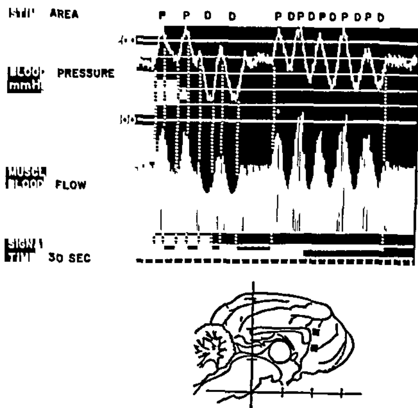


Fig 3 Cat 3 4 kg Chloralose Effects on arterial blood pressure and skeletal mus blood flow of bilateral, bipolar electrical stimulation (3 V, 3 msec, 35 imp /sec) of t pressor (P) and depressor (D) areas In the last part of the figure the electrodes were mov between the two areas during continous stimulation Note the pronounced engageme of the skeletal muscle vessels in both pressor and depressor reactions For further deta see Fig 2

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The variability in constrictor fibre responses that occasionally was obtaine upon topical stimulation of closely situated structures within the pressor area is illustrated in Fig 4 In A the pressor effect is accompanied by a

decreased intestinal blood flow implying a regional increase of flow resistance amounting to 50 per cent while there is a passive blood flow increase within the skeletal muscles with no significant change in regional flow resistance In B and C of Fig 3 which refer to a topical stimulation only 0 5 and 1 mm respectively ventrally to the point stimulated in A there occurs a considerable increase of flow resistance in both the intestines and the skeletal muscles However from an electrode position only 0 5 mm further ventrally (D) topical electrical stimulation causes more than a doubling of the flow resistance within the skeletal muscles while now a merely passive flow increase occurs within the intestines Though only exceptionally observed such findings seem to indicate the existence in this area of a functionally very differentiated arrangement so far as the neuron pools exercising excitatory effects on the vasoconstrictor fibre system are concerned

It should in this connection be mentioned that neurogenically induced changes in renal blood flow could sometimes be produced from the pressor area However generally the vessels of the kidney seemed to be unaffected while simultaneously recorded muscle blood flow indicated a marked vasoconstriction within this latter tissue

In the experiment illustrated in Fig 5 the pressor and depressor areas referred to above are stimulated alternately while blood pressure and muscle blood flow are recorded At the first two signals the pressor area (P) is excited electrically and at the following two signals the depressor area (D) The last part of the figure shows the results when the electrodes are slowly moved up and down along a dorso ventral line between the pressor and depressor areas during continuous electrical stimulation Blood pressure falls so elicited are always combined with an increased muscle blood flow and *vice versa* These observations demonstrate both the specificity of the responses induced from a given cortical area and the fact that the muscle blood vessels appear to be especially strongly affected

It was a regular finding in the present experiments that when the electrodes were placed in central parts of the pressor or depressor areas respectively electrical stimulation induced clearcut pressor and depressor effects These were independent of stimulation frequency or other stimulation characteristics except when stimuli of extremely high voltage were used when current spread entails excitation of extensive tissue areas When however the electrodes were placed in intermediate positions where the depressor and pressor areas appear to overlap the response type was sometimes found to be dependent on the stimulation frequency as in the experiment shown in Fig 6 In A and B of this figure point (22 19) within the depressor area is subjected to electrical stimuli of low (8 sec) and high (80 sec) frequencies During C

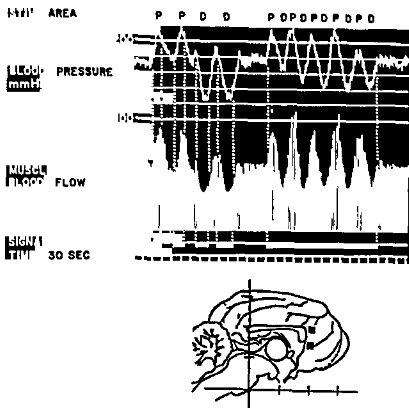


Fig 5 Cat 3 4 kg Chloralose Effects on arterial blood pressure and skeletal muscle blood flow of bilateral bipolar electrical stimulation (3 V, 3 msec 30 imp/sec) of the pressor (P) and depressor (D) areas In the last part of the figure the electrodes were moved between the two areas during continous stimulation Note the pronounced engagement of the skeletal muscle vessels in both pressor and depressor reactions For further details see Fig 2

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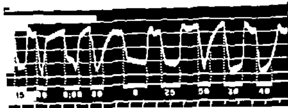


Fig 7 Cat 3 8 kg Chloralose The extent of the depressor responses when stimuli of different frequencies are applied to the depressor area at point (21 19) Note that sustained depressor responses can only be obtained with stimuli of lower frequencies (below 30—35 imp/sec) — For further details see Fig 2

to a rise of blood pressure while the 'inhibitory' cortical fibres had a relatively more powerful influence at lower frequencies

Fig 7 illustrates the correlation between stimulation frequency and the extent of the depressor responses when one and the same point (21, 19) within the cortical depressor area is electrically excited It is seen from this figure that even fairly low frequencies are able to induce powerful depressor effects sometimes rates as low as 2—3 impulses/sec (not shown in the figure) elicited significant blood pressure falls The correlation between the frequency and the extent of the elicited depressor response appears to form a hyperbolic curve with a steeply increasing response at lower rates and where almost maximal responses are reached at 20—30 impulses sec Further, at *these or lower frequencies it is possible to maintain the depressor response* almost unchanged for periods of several minutes At higher frequencies the depressor responses are often initially slightly greater than those obtained by 20—30 impulses sec but the effects then gradually subside in spite of continued stimulation and sometimes almost vanish

It was also considered to be of interest to examine the behavioural changes which occurred in conscious cats when points within the cingulate 'depressor area' were stimulated In *preliminary experiments* undertaken to attempt some correlation between somatomotor and the observed visceromotor responses electrodes were chronically implanted within this area in five cats Electrical stimulations of the 'depressor area' of the cingulate gyrus of these unanesthetized freely moving cats generally released a rather complex pattern characterized by an immediate cessation of spontaneous muscle activity, giving an impression that the animal had suddenly become frozen' By palpation of the skeletal muscles and by moving the limbs passively it was

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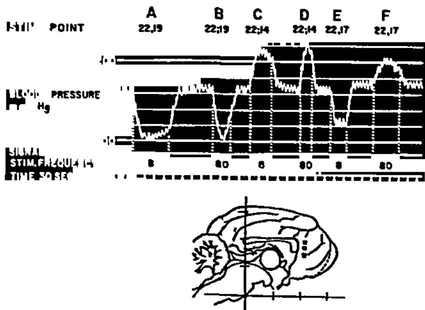


Fig 6 Cat 2 4 kg Chloralose Effects on arterial blood pressure of bilateral, bipolar electrical stimulation (4 V, 3 msec, 8 or 80 imp /sec) of the depressor area (A and B), of the pressor area (C and D) and at an intermediate position between these areas (E and F) Note that in the last electrode position the response is qualitatively frequency conditioned — For further details see Fig 2

and D the pressor area is excited with identical stimuli at point (22, 14) It is seen that pure depressor and pressor effects, appropriate to the points stimulated, are obtained whether the applied frequency is low or high However, in E and F, where the electrodes are placed intermediately between the pressor and depressor areas (22, 17), a low frequency stimulation produces a depressor effect while a high-frequency stimulation induces a pure pressor response from the same electrode position Other stimulation characteristics were kept the same Such frequency conditioned blood pressure responses were often observed when this particular region was stimulated

The most likely interpretation of such a phenomenon is that there exists a certain overlap between 'excitatory' and 'inhibitory' fibres within the different cortical areas studied, and that these fibres exhibit somewhat different frequency response characteristics with regard to their influence on sympathetic activity When the cortical structures of such intermediate zones were excited at high rates it appeared as if the excitatory fibres dominated the central influence on the lower sympathetic centres, leading

fairly undisturbed in superficial anesthesia Hence the cortically induced depressor or pressor effects are in a most unfavourable competitive position when facing the generally counteracting reflex adjustments in an anesthetized animal and they will necessarily be much weakened or possibly even completely masked under such circumstances

Thus many factors will tend to diminish the extent of influence of the studied cortical structures on the cardiovascular system Some of them depend on the anesthesia others are inherent in the type of technique employed Therefore there are reasons for assuming that in the intact organism a massive *physiological* excitation of the limbic autonomic structures might cause even more extensive cardiovascular shifts than those produced by topical cortical stimulation in anesthetized animals deprived of their homeostatic blood pressure reflexes It should be recalled (see Chapter I) that similar stimulations of the cingulate gyrus in e g conscious monkeys can cause a complete cardiac arrest by way of a cortical activation of the vagal heart fibres (SMITH 1945) This fact may represent one example of the power of these limbic autonomic centres so far as concerns the extent of their influence on the cardiovascular system

It might be questioned why on the whole anesthesia has been used in a study of the present type The position is however different from that when e g only a few easily available autonomic parameters such as the heart rate or the pupillary reactions are to be studied A detailed analysis of cardiovascular adjustments implying complex redistributions of blood flow necessarily demands extensive preparations and procedures for elimination of artefacts Apart from the obvious humane reasons general anesthesia is here necessary because otherwise the animals are easily brought into a shocklike condition The centrally induced visceromotor reactions and even the reflex cardiovascular control in general might otherwise in fact be more extensively disturbed than when the animals are kept in a state of carefully controlled superficial anesthesia This is presumably due to the intense abnormal activation of different peripheral receptors caused by the operative procedures Their profound afferent influence may severely disturb the functional balance of the highest nervous centres and often they cause marked inhibitions of sympathetic tone Such disturbing effects may be better kept under control by using moderate amounts of anesthetic agents.

These exploratory series of experiments suggest that there exist an autonomic representation of sympatho inhibitory neurons within the anterior part of the cingulate gyrus with its centre just rostral to the genu of the corpus callosum This depressor area is however not strictly circumscribed and in the ventral direction it partly overlaps with a pressor area situated

revealed that a very marked decrease of skeletal muscle tone occurred Provocation by *pulling a string snared around an ankle or the tail* — a procedure which normally produced vigorous protective gestures or even aggressive attitudes — was in most cases quite passively accepted as long as the stimulation continued In addition the stimulations mostly caused an immediate depression of the respiration also seen in anesthetized animals if cortical stimulations were performed before curarization Immediately upon cessation of the stimulation the animals started to move as if nothing had happened and showed *the same reactions to provocation as before the stimulations* The effects produced by stimulations of the cingulate depressor area in conscious animals which are in agreement with those reported in earlier studies (for ref see Chapter I) will be further extended in subsequent studies However these preliminary observations suggest that the inhibition of sympathetic activity caused by activation of the rostral cingulate gyrus *may normally be accompanied by an inhibition of somatomotor activity affecting both the control of the respiratory muscles and the skeletal muscles in general*

b Comments In the lightly anesthetized but otherwise essentially intact cat topical stimulation of rostral parts of the limbic system produced only moderate and transient blood pressure changes However when the range of influence of the limbic depressor and pressor areas on the cardiovascular system is considered it has to be borne in mind that a number of circumstances will *tend to diminish the extent of cortically elicited cardiovascular* responses in experiments of the present type Both the inhibitory and excitatory neurons within the areas studied appear to be scattered within a comparatively large tissue volume Therefore employment of the technique of discrete topical stimulation may activate only a fraction of the fibres that are normally engaged in the intact organism *Added to this is the fact that* the cortical neurons eliciting inhibitory or excitatory effects on tonic sympathetic discharge seem to be spatially intermingled to some degree as is illustrated in *e g* Fig *6 giving a tendency to diminish the extent of an induced* depressor as well as a pressor response In the normal reaction patterns of the intact animal on the other hand such crudely mixed activations of antagonistic neurons can hardly be expected to occur

In addition the *fact that the cerebral cortex forms* the part of the central nervous system which is most sensitive to anesthetic agents evidently implies that cortical autonomic effects can be expected to be severely depressed even by light anesthesia On the other hand the cardiovascular receptors which by wa of their bulbar reflex centres will damp or modify the influence of neurogenic adjustments *arising elsewhere will still exercise their influence*

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within the subcallosal region In the intermediate overlapping zone both depressor and pressor effects can be induced depending on the stimulation characteristics used

The vascular responses that can be elicited from the two areas seem to be mediated via the sympathetic vasoconstrictor fibres in such a way that from the depressor area an inhibition and from the pressor area an excitation of the tonic activity in the vasoconstrictor fibres can be produced There is no evidence that the sympathetic cholinergic vasodilator fibres should be engaged in any of these response patterns This preliminary experimental series already suggests that the different vascular regions are not equally affected when these limbic depressor and pressor areas are stimulated In particular the patterns that can be induced from the pressor area can be highly differentiated and complex and deserve individual study The depressor response pattern will be further dealt with in the subsequent chapters

In accordance with earlier investigations it has been confirmed that close to and partly coincident with the depressor area there is a cortical para sympathetic representation (see Chapter IV) This is of relevant interest since it is able to produce vagal bradycardia It is possible that this activation of the vagal heart fibres forms part of the normal depressor pattern elicited from this section of the cingulate gyrus The vagal fibres to the heart can be so strongly activated from this area that in monkeys (SMITH 1945 WARD 1948 KAADA 1951) a temporary arrest of the heart can be brought about Also in cats the effect can be considerable though not as marked as in the monkey (KAADA 1951)

Concerning the frequency response characteristics of the cingulate sym patho inhibitory fibres pronounced depressor responses can be elicited at rates below 5 impulses sec and about maximal effects are reached around 20—30 impulses sec At higher stimulation rates there is evidence of a gradual transmission failure of the sympatho inhibitory fibres This phenomenon of response escape might be due to the fact that the presumably fine calibre autonomic neuron links affecting the tonic activity of the vaso constrictor fibre system were forced to discharge at supraphysiological rates leading to a gradual *transmission failure due e g to synaptic fatigue* The escape phenomenon was scarcely due to fibre damage caused by the electrical stimulation *per se* as shifting the frequency to lower rates once again *led to a sustained depressor response Whatever the exact background* such findings suggested that the maximal discharge frequencies of the cortical sympatho inhibitory neurons were normally fairly low presumably *well below 20—30 impulses/sec* It appears as if the sympatho excitatory neurons may have a somewhat higher frequency optimum at least to judge from

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Concerning the frequency response characteristics of the cingulate sympatho-inhibitory fibres, pronounced depressor responses can be elicited at rates below 5 impulses/sec and about maximal effects are reached around 20—30 impulses/sec. At higher stimulation rates there is evidence of a gradual transmission failure of the sympatho-inhibitory fibres. This phenomenon of "response escape" might be due to the fact that the presumably fine calibre autonomic neuron links, affecting the tonic activity of the vasoconstrictor fibre system, were forced to discharge at supraphysiological rates, leading to a gradual *transmission failure due e. g. to synaptic fatigue*. The escape phenomenon was scarcely due to fibre damage caused by the electrical stimulation *per se*, as shifting the frequency to lower rates once again led to a sustained depressor response. Whatever the exact background, such findings suggested that the maximal discharge frequencies of these cortical sympatho-inhibitory neurons were normally fairly low, presumably well below 20—30 impulses/sec. It appears as if the sympatho-excitatory neurons may have a somewhat *higher frequency optimum*, at least to judge from

to allow a correct interpretation of the cortically elicited sympatho inhibitory pattern it was found essential to analyse in more detail the distribution of vasoconstrictor fibre discharge taking place when the medullary vasomotor centre was released from the damping influence of the baroceptors and or excited by intensified chemoreceptor activity This supplementary study which has previously been outlined in other connections (LOFVING 1961) will be briefly described in the last part of the of the present chapter as the results are important for the correct evaluation of the cortically induced depressor responses

1 The effect of electrical stimulation within the cingulate depressor area on four functionally different vascular regions and on heart rate

a Results As mentioned in the previous chapter it was easily demonstrated that there existed a powerful sympatho inhibitory representation in the more rostrally situated regions of the cingulate gyrus surrounding the genu of corpus callosum Stimulation within these anterior parts could produce very marked blood pressure falls in atropinized curarized animals as was al o evident from the exploratory experiments described in the previous chapter

Fig 8 demonstrates typical effects of such a topical stimulation where the systemic blood pressure *muscle* and *skin* blood flows are recorded simultaneously in an atropinized curarized cat kept under constant artificial respiration The initial blood pressure is high 220 mm Hg due to bilateral carotid occlusion and vagotomy and the blood pressure fall caused by the cortical stimulation (A) amounts to almost 100 mm Hg Despite this profound decrease of perfusion pressure blood flow in the skeletal muscles increases In fact the venous outflow from the muscle region is almost doubled during the stimulation compared with the prestimulation value and the regional peripheral resistance decreases by about 70 per cent of the initial value The skin blood flow of the paw on the other hand where in this experiment the pads with their arterio venous shunts were excluded from the circulation by tight ligatures shows a small decrease during the blood pressure fall but the regional peripheral resistance decreases by about 40 per cent of the prestimulation value In B of Fig 8 the same stimulation is repeated to demonstrate the uniformity of the response in one and the same experiment The perfusion pressure of the limb is then decreased to the level reached during the preceding stimulation by a partial occlusion of the abdominal aorta produced by a screw clamp During the next stimulation of the depressor area the inflow pressure to the limb is kept unchanged by adjusting the clamp (C in

CHAPTER IV

Analysis of the depressor response, elicited by stimulation of the rostral cingulate gyrus, with regard to the effects on different vascular regions and on heart rate

In the intact anesthetized animal electrical stimulation within the rostral cingulate gyrus was as mentioned in Chapter III able to produce only small and generally transient blood pressure falls This was mainly due to the fact that the elicited nervous patterns were markedly modified by the influence of blood pressure homeostatic factors like the baroreceptors This powerful reflex mechanism designed to keep the arterial blood pressure within rather narrow limits is relayed at the medullary level and is therefore probably far less affected by general anesthesia than the nervous activity within higher levels of the central nervous system Consequently the cortically induced effects on the peripheral vessels as well as on the parasympathectomized heart become small and so relatively difficult to analyze In order to facilitate a more correct evaluation of the cortically elicited peripheral cardiovascular changes it was found to be favourable to create a *relatively* constant level of activity within the medullary vasomotor centre In the majority of the experiments described in this chapter such a condition was established by a bilateral occlusion of the common carotids after the vagal and the aortic depressor nerves had been cut in the neck These procedures al o had the advantage with regard to the study of centrally induced depressor effects that an increased sympathetic activity was created for the bulbar vasomotor centre was then largely released from the powerful baroreceptor inhibition In other experiments the carotid arteries were not occluded but instead the carotid sinus region was infiltrated with a 2 per cent Xylocaine solution in order to block the influence of both the baro and chemoreceptor fibres The cortical stimulations could then exert their effect under circumstances where the tonic sympathetic activity was reasonably unaffected by the more important reflex modulating influences These results are reported in the first part of the present chapter

However in this state when the vasomotor centre was released from baroreceptor inhibitory activity it was soon apparent that the different vascular beds were affected by their constrictor fibres to a different extent *Therefore*

evident from the figure that the vessels of *both* tissues dilate as a result of the centrally induced inhibition of constrictor fibre activity, but the vasodilatation is much more pronounced within the skeletal muscles than in the skin During this stimulation period the flow resistance within the two tissues decreases by about 70 and 45 per cent of the initial values respectively Such a regional difference in extent of vasodilatation caused by the cortically induced inhibition of constrictor fibre tone, was regularly seen in the present experiments and was also apparent when the other vascular beds were compared with the muscle vessels as will be illustrated below In other experiments where the pad circulation with its arterio venous shunts, essentially subserving the hypothalamic heat loss centre was included in similar measurements blood flow generally decreased passively with no significant decrease of regional flow resistance during the cortically elicited depressor response This was evidently due to the fact that in most instances under the prevailing experimental conditions the tonic activity of the constrictor fibres controlling the specialized pad shunts was initially negligible, proved by the fact that cutting the vasoconstrictor fibres to this area did not increase the pad blood flow significantly On the whole it was a general experience that the constrictor fibres of these shunts appeared to be relatively little engaged in most cardiovascular reflex patterns with the exception of those subserving temperature regulation Therefore, since the pad circulation appeared to form a highly specialized vascular region the blood flow through the pads was generally excluded in the course of most experiments where the skin blood flow was measured

In Fig 9 a similar type of experiment is shown, but here the comparison is made between the vascular beds of the *skeletal muscles* and the *intestines* The extent of the cortically induced vasodilatation consequent upon an inhibition of constrictor fibre tone is even here more pronounced within the muscles and the decrease in regional flow resistance is about 60 and 30 per cent of the control value respectively These results too were typical for the relative extent of cortically induced vasodilatations within these particular vascular beds

In Fig 10 lastly a comparison is made between the effects of topical stimulation of the cingulate depressor area on the blood flow of the *skeletal muscles* and the *kidney* As was almost always the result in such experiments, the neurogenically conditioned flow resistance within the kidney remains practically unchanged during the topical stimulation of the cingulate gyrus, while the usual marked vasodilatation is seen within the skeletal muscles The fact that in experiments of this type a topical hypothalamic stimulation (not shown in Fig 10) was able to induce an intense renal vasoconstriction indicates

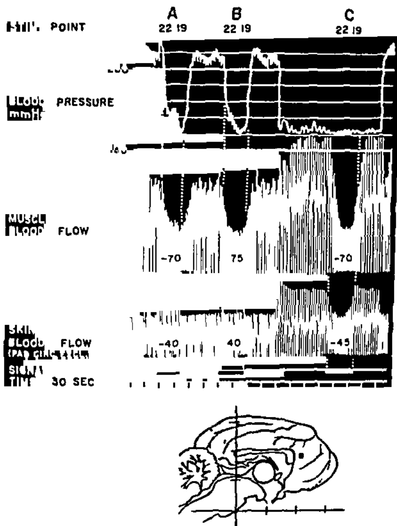


Fig 8 Cat 3 4 kg Chloralose Effects of bilateral bipolar electrical stimulation (4 V 3 msec 30 imp /sec) of the depressor area at point (22 19) on arterial blood pressure and on skeletal muscle and skin blood flows The arteriovenous anastomoses of the paw were excluded by ligatures around the pads The perfusion pressure to the regions studied is kept constant before during and after the third stimulation C Note that the vasodilatation within the skeletal muscle is by far more pronounced than that within the skin For further details see Fig 2

Fig 8) Under such circumstances the cortically induced vasoconstrictor fibre inhibition could exert its effect on the vascular regions studied at a constant and fairly normal perfusion pressure which allowed a more direct comparison of the sympatho inhibitory effects on the two vascular regions It is then

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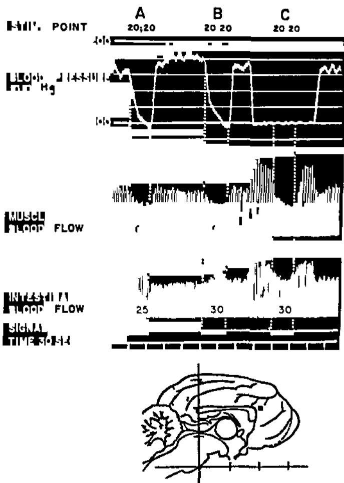


Fig 9 Cat 2 8 1 g Chloralose Effects of depressor area stimulation (3 5 V 3 msec 30 imp/sec) at point (20 20) on arterial blood pressure and on skeletal muscle and intestinal blood flows The perfusion pressure to the regions studied is kept constant before during and after the third stimulation C Note the quantitative difference in vasodilator response within the tissues studied — For further details see Fig 2

that the previous virtual absence of any neurogenic adjustments of renal blood flow was not due to any damage of the renal nerve supply

The almost constant finding in the present experiments of a pronounced neurogenic vasodilator effect on the muscle vessels might arouse the suspicion that the cholinergic sympathetic vasodilator fibres known to be distributed to the vessels of the skeletal muscles might have been activated from the cingulate gyrus in addition to the constrictor fibre inhibition In most experiments however cortical stimulations were performed both before and after

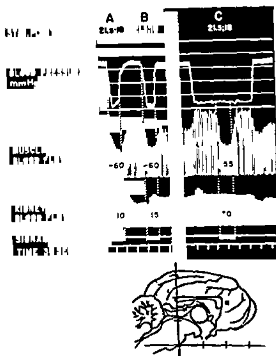


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intravenous administration of atropine in amounts sufficient to cause a complete peripheral block of the cholinergic vasodilator fibres A possible engagement of the dilator fibres would then have easily been revealed However even if large doses of atropine — more than 0 5 mg/kg bodyweight — were given this drug was never seen to change the magnitude or the type of the muscle blood flow increase following from stimulation of the cingulate depressor area On the other hand regional sympathectomy of the muscle vessels completely eliminated the muscle vasodilatation obtained coincidently with cortically induced blood pressure falls This fact together with the circumstance that the animals were completely curarized which rules out the possibility that any centrally induced activation of the skeletal muscles might have contributed to the vasodilator response makes it clear that the muscle

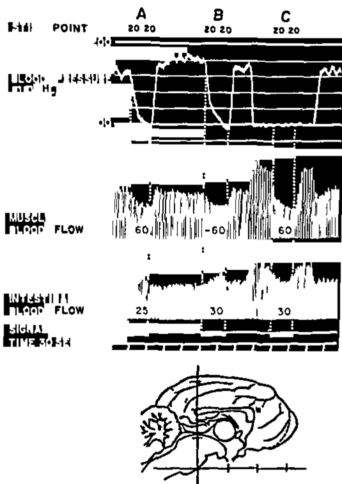


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depressor area for hypothalamic stimulations were able to produce marked constriction of the renal vessels

These results might at first sight suggest a rather selective inhibitory influence from the depressor area of the rostral cingulate gyrus affecting the tonic constrictor fibre activity predominantly in certain vascular beds However the cortically induced vascular effects were studied against a background of a comparatively high vasoconstrictor tone It is possible that the neuron pools controlling the different vascular regions studied were not equally excited under the control conditions If so even a generalized cortical inhibition of tonic sympathetic activity would cause only a minute dilatation where the prevailing tone was slight and a more pronounced one where constrictor fibre tone was intense

There are several data available concerning the characteristics of the VMC discharge and the modulating influence of the baro and chemoreceptors on single vascular beds (HEYMANS and NEIL 1958) However few if any attempts have so far been made to compare in detail to what extent different vascular regions are influenced by changes of the activity within the medullary vasomotor centre

From earlier data it could not therefore be judged whether it was the inherent tonic discharge of the vasoconstrictor fibres or the cortical inhibitory influence that was differentiated or both In a separate series of experiments attempts were therefore made to ascertain if regional differences in the rate of vasoconstrictor fibre discharge might exist during the state of increased vasoconstrictor activity that was used as the control level

2 Analysis of the effect on different vascular beds produced by shifts in baro and chemoreceptor activity

A relatively constant vasomotor centre activity was established by bilateral carotid occlusion after cutting the vagal and aortic depressor nerves The increased activity of the bulbar vasomotor centre so induced was probably exclusively due to a decreased baroreceptor inhibition but a concomitant excitation via the chemoreceptor fibres could by no means be excluded (LANDGREN and NEIL 1951 CHUNGCHAPOEN et al 1952) Both these reflexogenic mechanisms could be expected to induce a differentiation of the tonic constrictor fibre discharge They might even do so in principle different directions when in various experiments the constrictor fibre discharge pattern could be expected to vary depending on which of the two mechanisms was mainly responsible for the prevailing tonic sympathetic activity On the other hand if

vasodilation really is caused entirely by an inhibition of constrictor fibre tone The reason why this effect is especially marked within the muscle will be discussed below

The heart rate was counted at the beginning of most of the experiments before and after vagotomy or atropinization and in connection with the cortical stimulations In some experiments the changes in heart rate were directly recorded as described in Chapter II An excitation of the rostral cingulate gyrus was found to decrease the heart rate up to about 40 per cent of the control value as long as the vagal nerves were intact or atropine was not given Considering the fact that the extent of reflex bradycardia caused *e g* by baroreceptor activation is generally moderate in cats this heart rate decrease can be considered to imply a relatively marked central inhibitory effect on the heart pacemaker After elimination of the parasympathetic influence on the heart by cutting the vagal nerves or by administration of atropine cortical stimulation was still able to decrease the heart rate by about 10 per cent occasionally even up to 20 per cent in experiments when the carotid occlusion appeared to intensify the tonic sympathetic activity more markedly These residual inhibitory effects on heart rate were independent of possible reflex shifts in the secretion of the adrenal medullae They occurred promptly as a result of the cortical stimulation and also in experiments where the adrenal glands had been denervated and must be ascribed to a centrally induced inhibition of the tonic activity of the sympathetic acceleraus fibres

b Comments From the experimental data described above it was evident that from the depressor area situated in the rostral cingulate gyrus an inhibition of the tonic activity of the vasoconstrictor and the accelerans fibres could be produced associated with an activation of the vagal fibres to the heart However the inhibition seemed to affect the vessels of the skeletal muscles to a far greater extent than the vessels of the intestines and the skin as judged from the vasodilator responses while the effect on the renal vessels and the specialized pad vessels was generally negligible This characteristic pattern of cortically induced vasodilatations was essentially the same as long as the electrodes were situated within the cingulate depressor area The effects varied quantitatively but not qualitatively if the electrode position was slightly shifted These regional differences in extent of the cortically induced vasodilatation could not be ascribed to any other mechanisms such as an activation of the sympathetic vasodilator fibres Nor could they be due to any accidental damage of the sympathetic nerves in regions like the kidneys where the vessels were largely unaffected by the stimulation of the cingulate

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Therefore at the very beginning of this investigation a separate series of experiments was performed to examine these possibilities The results of this study have been briefly outlined in another connection (LOFVING 1961) The essential results will however be reported here as they are of importance for the evaluation of the cortical sympatho inhibitory responses

a Results By adjustments of a screw clamp placed around the innominate artery (for method see GROOM *et al* 1959 1961) the pulse pressure within both the common carotid arteries was markedly reduced while the mean carotid pressure was well maintained The increased vasomotor activity so induced was supposed to be due to a selective decrease in the baroreceptor fibre discharge as the perfusion pressure of the carotid bodies was essentially unaffected so that their receptors would be unlikely to be activated Fig 11 is taken from such an experiment where a blood pressure elevation of 80 mm Hg is obtained as a response to partial occlusion of the innominate artery The systemic blood pressure is recorded and the mean blood pressure cephalad to the partial occlusion of the innominate artery (not shown in the figure) is controlled to ensure that it is essentially unchanged During A there is a marked decrease of muscle blood flow in spite of the reflex rise in pressure while at the quantitatively almost identical reflex blood pressure rise in D and F performed somewhat later in the experiment renal blood flow increases During B and E the same manoeuvre is performed but to facilitate a comparison between the neurogenic adjustments of muscle and renal blood flows the perfusion pressure to the two tissues is kept constant by partial occlusion also of the abdominal aorta It is then seen that selective elimination of the baroreceptor influence causes a marked vasoconstriction within the skeletal muscles while the renal vessels remain completely unaffected After this sequence of observations the sympathetic nerves to the two tissues are cut centrally and subjected to direct peripheral stimulations at supramaximal intensities but at graded frequencies (C and G) in order to evaluate the average constrictor discharge rate in the two vascular beds just before and during the elimination of the baroreceptor influence as is illustrated in B and E respectively Accordingly the resting vasoconstrictor fibre tone of the muscle vessels is somewhat below 1 impulse/sec increasing to about 2—4 impulses/sec when the VMC is released from the inhibitory baroreceptor influence The constrictor fibres to the kidneys appear to be virtually inactive during resting conditions and are not noticeably activated when the baroreceptor influence is eliminated in E No increase of flow resistance takes place here while a direct stimulation of the renal vasoconstrictor fibres at 1 impulse/sec increases renal flow resistance by about 20 per cent

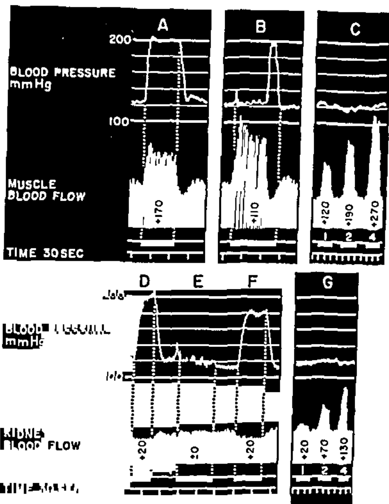


Fig 11 Cat 2 6 kg Chloralose A, D and F demonstrate the effects on arterial blood pressure and on skeletal muscle and kidney blood flows of a decreased baroreceptor activity, induced by 'elimination' of the pulse pressure within both the carotid sinus regions In B and E the same manoeuvre is repeated, but now (except from a short period in B) a partial occlusion of the abdominal aorta was induced to keep the perfusion pressure to respective vascular beds constant during the blood pressure rise After B and F the sympathetic nerves to the two regions were cut and during C and G the distal ends are subjected to supramaximal stimulation with 1, 2 and 4 imp /sec

A comparison between the blood flow recordings in B and C indicates that during B the average vasoconstrictor fibre discharge to the muscle vessels is about 2—4 imp /sec, while the corresponding rate of discharge in the renal vasoconstrictor fibres (to judge from E and G) is below 1 imp /sec

a decreased baroreceptor and an increased chemoreceptor activity produced essentially similar efferent discharge patterns, the effect on different vascular regions caused by a baroreceptor elimination would merely be intensified, but hardly changed in its pattern, by an additional chemoreceptor activation

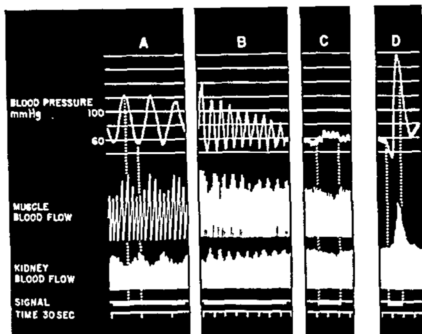


Fig 12 Cat 2 6 kg Chloralose Skeletal muscle and kidney blood flows during Mayer waves induced by carotid occlusion after previous bleeding During A the recording is performed with high paper speed Between A and B the sinus nerves on both sides were blocked with a 2 per cent Xylocaine solution which gradually diminished the blood pressure fluctuations Four minutes later C bilateral occlusion of the common carotids is not able to produce any Mayer waves In D the anterior hypothalamus is electrically stimulated to test the intactness of sympathetic nerves to the kidney Note that during Mayer waves concomitantly with a blood pressure rise the muscle blood flow decreases while the renal blood flow increases — Initially in the experiment the vagi and the aortic depressor nerves were cut in the neck and the reflex release of catechol amines from the adrenal medullae eliminated

fibre distribution or to differences in effector sensitivity It is known from earlier studies in this laboratory (CELANDER and FOLKOW 1953 CELANDER 1954) repeatedly confirmed in the present study, that at one and the same discharge rate the renal vessels are almost as much constricted as the muscle vessels and the cutaneous vessels even more so It therefore appeared to be clear that the observed regional differences, calculated in terms of peripheral resistance changes must be due to regional differences in the discharge rate, and/or in fibre recruitment of the vasomotor neuron pools controlling the vessels of the different tissues

Similar experiments when the intestinal and cutaneous blood flows were observed during decreased baroreceptor activity revealed that there was a significant increase of the sympathetic discharge to these regions normally corresponding to about 1—2 impulses/sec The intestinal vessels were however usually more affected than those of the skin The reflex effect in the latter region varied somewhat from one animal to another depending on the initial tone of the vessels which was presumably related to heat regulating centre influences on their constrictor fibres It was however, always far less than that of the skeletal muscle especially considering the fact that the cutaneous vasoconstrictor fibres exert a particularly dominating influence on their *subordinated vessels for any given discharge rate*

Fig 12 is taken from an experiment where the effect of induced 'Mayer waves' on the same two regional flows are studied These big fluctuations in *systemic blood pressure which are caused by periodic, intense discharges of* the chemoreceptors (ANDERSSON, KENNEY and NEIL 1950) can be induced if the common carotids are occluded after the animals have been bled Thus in this case the baroreceptor influence was largely eliminated and in addition the chemoreceptors were strongly activated in rhythmic bursts It is seen from A in Fig 12 which is performed with high paper speed that an *elevation* of the systemic blood pressure corresponds to a *decrease* in the muscle blood flow while simultaneously there is a passive *increase* of the renal blood flow Between A and B the two sinus nerves were blocked by infiltration of the carotid sinus regions with a 2 per cent Xylocaine solution which gradually diminished the response as is illustrated in B of Fig 12 In C performed 4 min later the carotid arteries are occluded but due to block of the sinus nerves no Mayer waves are induced Hypothalamic stimulation (D) on the other hand is able to induce a considerable renal vasoconstriction proving that the vasoconstrictor fibres to this region were essentially intact Similar observations on the intestinal and cutaneous blood flows revealed that the induced Mayer waves distinctly affected the nervous tone of the vessels in these regions but to a far less extent than that of the skeletal muscle vessels Thus addition of chemoreceptor activation to the effect of elimination of the baroreceptor influence did not produce any entirely different efferent discharge pattern but rather an intensification of that caused by an elimination of the baroreceptor influence

b Comments From this series of experiments it is evident that the increased vasoconstrictor fibre tone caused by a decreased baroreceptor activity did not affect the vessels in the four different tissues studied to an equal extent This could not be ascribed to regional differences in the extent of constrictor

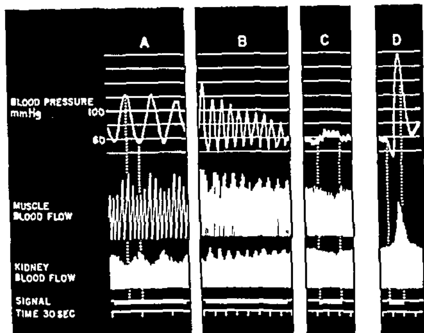


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It was regularly seen in these experiments that the vessels of the skeletal muscles were far more affected by changes in baroreceptor activity than those of other tissues studied, whether such changes were produced by alterations of the carotid pulse pressure, mean blood pressure or simply by pulling on the occluded common arteries. The *vasoconstrictor effects in the pads of* the paws, with their abundance of arteriovenous anastomoses were, on the other hand, especially weak and often almost absent under such circumstances. *Even if the pad circulation was obstructed in the skin blood flow measure*ments the neurogenic effects were nevertheless always weaker within the skin than within the skeletal muscles or the intestines.

The constrictor fibre response pattern caused when a *chemoreceptor stimulation* was added to the effect of a baroreceptor elimination, was not in principle changed but caused only some intensification of the response induced by a selective baroreceptor elimination. It resulted in a vasoconstriction which was quite pronounced within the skeletal muscles, distinct but comparatively moderate within the intestines, small within the skin, while the renal vessels were generally only insignificantly affected.

With respect to the pattern of vasoconstrictor fibre discharge the results suggested that the constrictor fibres to the muscle vessels were strongly affected, those to the intestinal vessels moderately so, while those supplying the cutaneous and the renal vessels were slightly or even insignificantly activated when the vasomotor centre was released from the baroreceptor inhibition and/or excited by the chemoreceptors, other things being unchanged.

However, there occurred occasionally divergences in the responses insofar as changes of the baro- and chemoreceptor activity sometimes did affect e. g. the renal vessels more intensely. Is is also well known that in several experimental conditions extensive reflex renal vasoconstrictions are seen. A reasonable explanation of these somewhat varying results has been presented in a recent paper (FOLKOW, JOHANSSON and LÖFVING 1961). In this study evidence was presented which seemed to suggest that the different autonomic neuron pools controlling the functionally different vascular beds exhibit different levels of excitability or thresholds. The vasoconstrictor neurons *controlling* the muscle vessels can be considered to have low thresholds, exhibiting a resting tonic discharge which is promptly intensified if the central excitatory state is increased by addition of even slight excitatory influences. *Those controlling e. g.* the renal vessels, on the other hand, can be considered to be high threshold with no significant tonic discharge under resting conditions. If however such high threshold neuron pools are exposed to an intense excitatory *influence*, *as in asphyxia*, or when subliminal excitatory influences

are allowed to act concomitantly these neurons also can become markedly activated This is seen for instance when an elimination of the baroreceptors is combined with a stimulation of nociceptive fibres when each individual excitatory influence on the sympathetic centres may be too weak to affect the renal vasoconstrictor fibres to any significant extent (JOHANSSON 1961)

Thus moderate quantitative differences in the excitability levels of the different sympathetic neuron pools appear to constitute an important basis for the elicitation of often highly differentiated constrictor fibre discharge patterns but allow also for more diffuse mass activations in *e g* intense asphyxia

The data presented in the two sections of this chapter thus made it clear that it is the basic pattern of tonic constrictor fibre discharge that is differentiated at least under certain circumstances such as those created in the present experiments The cortically induced sympatho inhibitory effects appear to be fairly diffuse and undifferentiated affecting all regions where there exists any tonic sympathetic activity that can be inhibited This is further supported by the results illustrated in Fig 16 in Chapter V It is here seen that the cortical depressor area also exerts an inhibitory influence on the constrictor neurons controlling the renal vessels *provided only that* these neurons have been initially excited to exhibit a tonic discharge

CHAPTER V

The centrifugal projections of the sympatho-inhibitory neurons of the rostral cingulate gyrus and their relationship to some reflexly induced inhibitions of tonic sympathetic activity

This chapter is concerned with three separate series of experiments which are referred to below as (a) (b) and (c)

Although a number of studies has been performed with respect to the centrifugal projections of the cingulate gyrus (KAADA 1951 pp 146—160) little is known about the descending pathways mediating the cardiovascular changes Recently FOLKOW JOHANSSON and ÖBERG (1959) were able to localize a powerful sympatho inhibitory area in the rostral hypothalamus near the anterior commisure The proximity of the rostral cingulate gyrus and the anterior hypothalamus aroused the suspicion that the sympatho inhibitory neurons from the cingulate depressor area might project to this sympatho inhibitory *structure the more so as the cardiovascular depressor* patterns elicited from the two structures appear to be very similar In a series of experiments the effect of localized electrolytic lesion of this hypothalamic area upon the cortically induced depressor effects has therefore been studied (a)

In another series of experiments a comparison has been made between the cortically induced sympatho inhibitory patterns and those produced by excitation of the baroreceptors and by direct stimulation of the medullary depressor area Such an analysis might reveal whether these inhibitory reactions are mediated by a similar possibly identical mechanism Therefore the cardiovascular sympatho inhibitory patterns induced by mechanical excitation of the carotid baroreceptors by electrical stimulation of the vagal nerves in afferent direction and by topical stimulation within the medullary depressor area were compared with the effect of topical stimulation of the cingulate depressor area (b)

In addition it was thought to be of interest to investigate whether there existed a more close anatomical relation between the depressor area of the cingulate gyrus and the medullary vasomotor centre *A priori* it is possible that the descending inhibitory pathways entirely bypass the medullary vaso

motor centre to exert a direct inhibitory effect on the autonomic final common path — the preganglionic neurons in the spinal medulla — but they might also exercise their effect via the medial portion of the medullary vasomotor centre the so called depressor point or depressor area (for literature see *e g* UVNAS 1960) If the latter alternative is true this would lend additional support to the view that the reflex inhibition of sympathetic tone elicited from the different cardiovascular receptors via the bulbar va o motor centre and the cortically induced inhibitions of sympathetic activity are both mediated via a common bulbar structure The cortically and reflexly induced depres or effects were therefore studied before and after localized lesions of the mentioned medullary structures (c)

Resu lts (a) In five cats anesthetized and prepared as described in Chapter II the effects on the blood pressure of topical stimulation of the rostral cingulate gyrus were studied before and after localized electrolytic lesions in the sympatho inhibitory area of the anterior hypothalamus Electrodes were first so placed within the cingulate depressor area as to produce a marked depressor effect on stimulation T vo concentric electrodes were then placed bilaterally in the hypothalamic sympatho inhibitory area When both the cortical and the hypothalamic electrodes had been correctly placed as tested by the marked depressor responses elicited by electrical stimulation in the atropinized vagotomized animal an electrolytic lesion was produced in the hypothalamic depressor area For this purpose the actual hypothalamic region was subjected for 2 minutes to a constant d c of about 10 mA During this procedure the electrodes were slowly moved up and down about one mm dorsally and ventrally to the point from which maximal depressor responses had earlier been elicited The damage produced was fairly localized as electrical stimulation in different points situated only 1—2 mm from the stated elect rode positions was able to induce blood pressure effects as pronounced as before the lesions had been produced The depressor responses elicited by stimulation in the cingulate depressor area were found to be markedly de pressed or abolished by these topical hypothalamic lesions Further in one experiment where attempts were made to induce graded very small hypothalamic lesions it was possible to eliminate almost completely the blood pressure decrease which was produced by topical stimulation of the most dorsal section of the cortical depressor area When the cortical electrodes were moved only 2 mm ventrally a marked blood pressure decrease could again be elicited by cortical stimulation which in turn could be eliminated by producing an additional hypothalamic lesion some mm ventrally to the previously produced lesion

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thalamic electrolytic damage was now increased in extent by moving the electrode up and down for some millimeters on both sides during a continuous d c of 10 mA Another five minutes later the same cortical point (21 18) is again stimulated Despite repeated stimulations of the cingulate depressor area at various frequencies and at increased voltage only a minute blood pressure fall can be evoked (D) The fact that topical stimulations within the subcallosal pressor area (not shown in the figure) were still able to produce fairly marked pressor responses indicated that the elimination of the depressor effect was by no means due to a generalized decrease of cortical excitability

Results (b) In a series of 10 experiments the effects of *different* types of sympatho inhibitory stimuli were observed with respect to their effect on arterial blood pressure and on blood flows in two regions analogous except for the fact that the vessels of one of them had been acutely sympathectomized In this way the effects on the blood flow in the skeletal muscles were studied in 3 experiments in the kidneys in 3 experiments and in the skin in 2 experiments Lastly in 2 experiments the effects of sympatho inhibitory stimulations were studied on the intestinal blood flow before and after acute sympathectomy of the intestinal blood vessels

Fig 14 shows an experiment where the *skeletal muscle* blood flow is recorded both in the right and left calves where the vessels of the left calf had been acutely denervated by extirpation of the left abdominal sympathetic chain at the level of the third to the fifth lumbar vertebras Initially in this experiment the animal had been atropinized and curarized after which the common carotids had been occluded and the vagal nerves cut A comparison of the two flow recordings where initially the ordinate heights had been so adjusted that they directly reflected the difference in flow resistance between the innervated and denervated vascular beds makes it obvious that the blood flow resistance is more than three times higher in the innervated calf muscles than in those acutely sympathectomized The figure also illustrates how the animal is subjected to different types of sympatho inhibitory influences induced during a period of a steady occlusion of the common carotids By careful gradation of the different inhibitory stimuli the induced blood pressure decreases are adjusted to about the same order of magnitude to *facilitate* a comparison between the extent of the induced vascular effects It is then seen from the figure that the relation between the blood pressure fall and the decrease of flow resistance in the normally innervated vessels is quantitatively very similar and is independent of the way in which the depressor effect is produced This was a regular finding in this type of experiment In A of Fig 14 the depressor area of the cingulate gyrus is topically stimulated in

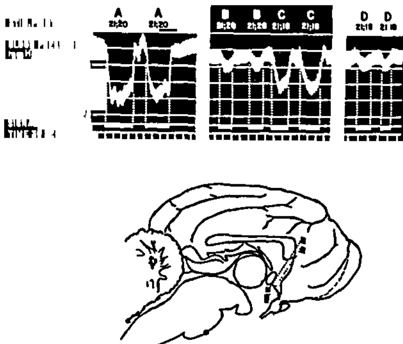


Fig 13 Cat 2 5 kg Chloralose Effects on arterial blood pressure of bilateral bipolar electrical stimulation of the depressor area before and after localized electrolytic lesions of the sympatho inhibitory area in the anterior hypothalamus In A point (21 20) is stimulated (3 V 3 msec 30 imp /sec) Between A and B a small electrolytic lesion was produced in rostral hypothalamus Five minutes later the stimulations of point (21 20) are repeated (B) with unchanged stimulation characteristics The electrodes were then moved 2 mm ventrally to point (21 18) and electrical stimuli (3 V 3 msec 30 imp /sec) applied The hypothalamic lesion was now increased somewhat in extent between C and D Five minutes later point (21 18) is again stimulated (3—7 V 3 msec 20—60 imp /sec) Note that the cortically induced depressor responses are almost completely abolished by the hypothalamic lesions For further details see Fig 2

Fig 13 shows a record from the experiment quoted above Stimulations of the rostral cingulate gyrus (21, 20) cause as usual a marked depressor response amounting to 60 mm Hg which is demonstrated in the first two tracings (A) The hypothalamic sympatho inhibitory centre was then bilaterally damaged as described above Five minutes later the stimulations are repeated (B) with the cortical electrodes in the same position using the same stimulation characteristics The induced depressor effects are now considerably reduced being only of the order of 15 mm Hg When however the cortical electrodes are moved 2 mm ventrally (21, 18) cortical stimulations again produce a prompt and rather marked blood pressure fall (C) The hypo

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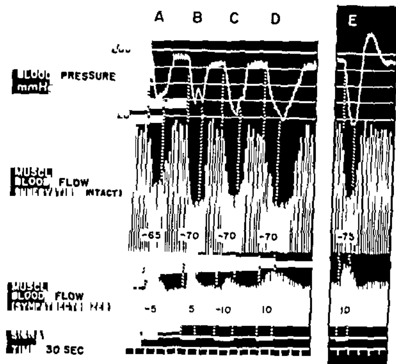


Fig 14 Cat 3 2 kg Chloralose Effects of different depressor stimuli on arterial blood pressure and on blood flows in two analogeous skeletal muscle regions one of which was acutely sympathectomized

A Cortical stimulation (3 V 3 msec 20 imp /sec) within the depressor area at point (21 20)

B Mechanical stimulation of the carotid baroreceptors caused by rhythmic pulling on the occluded common carotids

C Electrical stimulation (4 V, 5 msec 40 imp /sec) of the right aortic nerve

D Similar stimulation of the central end of the cut left vagus

E Topical stimulation (0 6 V, 2 msec 70 imp /sec) within the depressor area of the medullary vasomotor centre

Note that neurogenically induced vasodilatations of approximately the same magnitude are obtained as responses to the different inhibitory stimuli The blood flow changes in the sympathectomized region are essentially passive For further details see Fig 2

B the carotid baroreceptors are mechanically stimulated by rapidly repeated pulling on the occluded carotid arteries at a rate of about 150/min In C the central end of the cut right aortic nerve is stimulated in D that of the left vagal nerve and in E lastly a topical stimulation of the medullary 'depressor area' is performed It is also seen from the figure that the effects on the blood flow in the sympathectomized muscle region are essentially passive, being only to some extent adjusted by the local mechanisms responsible for the 'autoregulatory changes of vascular tone that take place normally

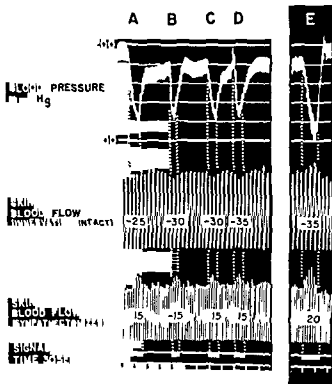


Fig 15 Cat 3 4 kg Chloralose Effects of d fferent depressor stimuli on system c blood pressure and on venous outflows from two analogeous skin regions where the vessels on one s de were sympathectomized Pad circulation was excluded
A Rhythmic pull ng on the occluded common carotid arteries
B and C Cort cal stimulation (3 V 3 msec 30 imp /sec) at point (72 18)
D Stimulation (3 V 5 msec 40 imp /sec) of the central end of the cut left vagus
E St mulation (0 5 V 3 msec 70 imp /sec) with n the depressor area of the medullary vasomotor centre

Note that the d fferences in peripheral resistance changes in the two regions are comparably small For further details see F g 2

within the muscle vascular bed (see *e g* FOLKOW and LOFVING 1956) If the perfusion pressure to the two limbs was equilibrated (*not shown in the figure*) during the depressor stimulations blood flow in the innervated limb increased markedly while that in the denervated limb remained unchanged

Fig 15 is taken from a comparable experiment where the blood flow changes are recorded from two *precisely identically* prepared *skin* regions of the hind paws except that the vessels of the *left* limb (lower blood flow tracing) had been acutely sympathectomized Initially the bulbar vasomotor

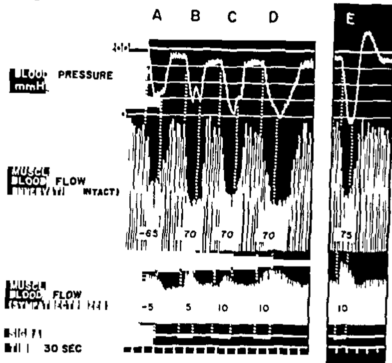


Fig 14 Cat 3 kg Chloralose Effects of different depressor stimuli on arterial blood pressure and on blood flows in two analogeous skeletal muscle regions one of which was acutely sympathectomized

A Cortical stimulation (3 V 3 msec, 20 imp /sec) within the depressor area at point (21 20)

B Mechanical stimulation of the carotid baroreceptors caused by rhythmic pulling on the occluded common carotids

C Electrical stimulation (4 V 5 msec 40 imp /sec) of the right aortic nerve

D Similar stimulation of the central end of the cut left vagus

E Topical stimulation (0 6 V 2 msec 70 imp /sec) within the depressor area of the medullary vasomotor centre

Note that neurogenically induced vasodilatations of approximately the same magnitude are obtained as responses to the different inhibitory stimuli The blood flow changes in the sympathectomized region are essentially passive For further details see Fig 2

B the carotid baroreceptors are mechanically stimulated by rapidly repeated pulling on the occluded carotid arteries at a rate of about 150/min In C the central end of the cut right aortic nerve is stimulated in D that of the left vagal nerve and in F lastly a topical stimulation of the medullary 'depressor area is performed It is also seen from the figure that the effects on the blood flow in the sympathectomized muscle region are essentially passive, being only to some extent adjusted by the local mechanisms responsible for the autoregulatory changes of vascular tone that take place normally

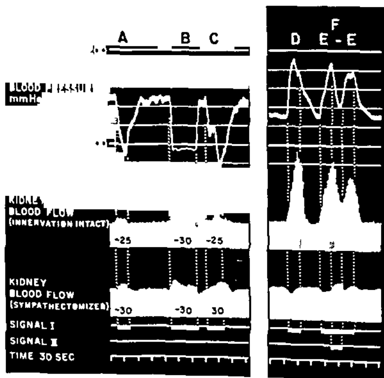


Fig 16 Cat 2 8 kg Chloralose Venous outflows from both kidneys one of which was sympathectomized

A Cortical stimulation (3 5 V 3 msec 40 imp/sec) at point (21 19)

B Passive decrease of the perfusion pressure to the kidneys produced by partial occlusion of the abdominal aorta

C Rhythmic pulling on the occluded common carotid arteries

D Electrical stimulation (2 V 3 msec 60 imp/sec) within the anterior hypothalamus

E Electrical stimulation of the central end of the cut sciatic nerve during which (in F) a cortical stimulation (4 V 3 msec, 40 imp/sec) at point (20 19) is induced

During A B and C the blood flow changes in the kidney with intact vasomotor innervation are almost identical to those in the sympathectomized one This was apparently due to the absence of an ... not ... In D and E the ... strated Note ... inhibited by s... ...cingulate depressor area F — ... is promptly reflex release of adrenal medullary hormones was prevented For further details see Fig 2

centre was released from the baroreceptor inhibitory influences as described above A comparison of the flow resistance of the two sides as judged from the heights of the ordinates makes it clear that the neurogenically induced changes in peripheral resistance are not so pronounced in the skin as in the skeletal muscle vessels being here of the order of only some 30 per cent of the initial value When different types of inhibitory influences are induced as in the preceding figure the relationship between the differently induced blood pressure fall and the decrease of resistance in the innervated cutaneous vascular bed is remarkably constant As usual the blood flow in the sympathectomized skin vessels follows in a more passive way the changes in perfusion pressure

Fig 16 illustrates a similar experiment in which the carotid arteries were occluded throughout the recording Here the blood flow changes within an innervated and an acutely sympathectomized *kidney* are simultaneously recorded while the sympathetic tonic discharge of the animal is exposed to inhibitory influences In contrast to the results obtained in the two previously described experiments there is practically no initial difference in flow resistance between the innervated and denervated kidney Under similar conditions the flow resistance in the muscles is generally increased 3–4 times as compared with a sympathectomized muscle region Further when exposed to the inhibitory stimulations the blood flow changes in the kidney with the intact sympathetic innervation are almost identical to those of the sympathectomized kidney A calculation of the flow resistance associated with the cortical stimulation (A) and the activation of the carotid baroreceptors (C) shows 25–30 per cent decrease but the same resistance decrease is seen also in the completely denervated kidney Moreover if the perfusion pressure is passively decreased by partial occlusion of the abdominal aorta (B) the decrease in flow resistance within the two kidneys is of the same order These decreases in flow resistance are obviously therefore a consequence of the blood pressure fall *per se* and merely reflect the well known autoregulation of the renal blood flow resistance The results thus suggested that even pronounced sympatho inhibitory influences did not affect the renal flow resistance via the renal vasoconstrictor fibres simply because these fibres did not exhibit any significant tonic activity that could be inhibited under the prevailing conditions The blood flows in the innervated and the sympathectomized kidney were so similar that it might well be suspected that the vasoconstrictor fibres of the innervated kidneys had been damaged during the preparation This was however not the case as a topical stimulation within the anterior hypothalamus (D) increases the flow resistance within the innervated kidney some 3–4 times As the adrenal glands were denervated the intactness of the sympathetic innervation of the kidney is therefore

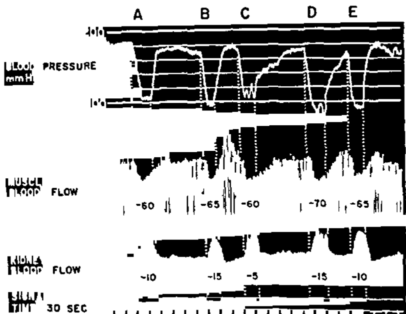


Fig 17 Cat 3 0 kg Chloralose Effects of different sympatho inhibitory stimuli on the blood flows in muscle and kidney both with intact vasomotor innervation
A Cortical stimulation (3 5 V 3 msec 30 imp /sec) at point (21 19)
B and E Stimulation (0 5 V 2 msec 60 imp /sec) within the depressor area of the medullary vasomotor centre
C Rhythmic pulling on the occluded common carotid arteries
D Stimulation (4 V 2 msec 40 imp /sec) of the proximal end of the cut left vagus
The figure illustrates that the vascular responses *in respective* regions are essentially the same independent of the type of the sympatho inhibitory stimulus applied Renal blood flow changes are essentially passive due to the absence of any renal vasoconstrictor tone For further details see Fig 7

types of baroreceptor fibres were observed before and after an electrolytic damage to the medullary depressor area' The lesion was, thus performed in the midline at the lower part of the fourth ventricle The animals were prepared as described in Chapter II with a concentric electrode orientated for topical stimulation of the medullary depressor area to be later used to produce an electrolytic lesion of this area

Fig 18 illustrates an experiment of this type It is seen that a pronounced blood pressure fall is obtained on stimulation of the cingulate depressor area

well established The results of hypothalamic stimulation also confirm that the denervation of the other kidney is complete However if the constrictor fibres of the renal vessels are made to discharge (E) by an intense afferent stimulation of nociceptor fibres (JOHANSSON 1961) a stimulation of the cingulate depressor area is able to inhibit this renal vasoconstrictor fibre activity (F in Fig 16) This experiment thus makes it clear that the cingulate sympatho inhibitory neurons are able to exert an effect even on the activity of the renal vasoconstrictor fibres provided only that a constrictor fibre tone exists

Similar studies of the vessels of the *intestines* where inhibitory stimulations were performed before and after sympathectomy revealed that in vagotomized atropinized animals the vessels of the jejunum generally exhibited a constrictor fibre tone intermediate between that of the muscle vessels and the cutaneous vessels when the carotid arteries were occluded Similarly when still innervated the intestinal blood vessels dilated moderately and equally in response to different types of depressor stimuli It was however never observed under such circumstances that the intestinal vessels were more strongly affected than the muscle vessels

In Fig 17 the effects of different inhibitory stimuli on the blood flow of a skeletal muscle region and a kidney both with an intact vasoconstrictor fibre innervation are illustrated Before the records shown in this figure were obtained the carotid arteries were occluded this procedure markedly increased the resistance in muscle blood vessels whilst that within kidney vessels was barely affected As shown in the tracing the animal is then exposed to the usual series of different types of sympatho inhibitory influences Attempts are made to gradate the differently produced depressor effects so that the blood pressure falls are of about the same magnitude The dilator effect on the skeletal muscle vessels consequent upon the inhibition of constrictor fibre tone is here of the same order whether the inhibition is induced by stimulation of the cortical depressor area (A) by stimulation of the medullary depressor area (B and E) by mechanical activation of the carotid baroreceptors (C) or by afferent stimulation of the cut left vagal and aortic depressor nerves (D) A similar comparison of these depressor effects on the renal blood flow reveals that this is merely passively decreased to the same extent independently of the way in which the depressor effect is produced Evidently there is no prevailing renal vasoconstrictor fibre activity which can be inhibited As usual it was ascertained by a hypothalamic stimulation that the renal vasoconstrictor fibre supply was intact (not shown in the figure)

Results (c) In a series of 7 experiments the depressor effects induced by stimulation of the rostral cingulate gyrus and by activation of the different

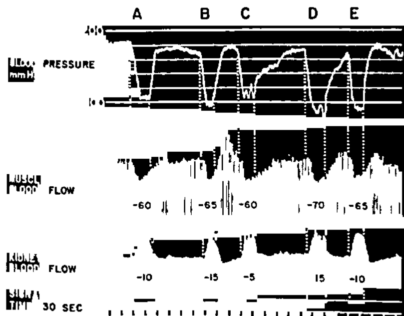


Fig 17 Cat 3 0 kg Chloralose Effects of different sympatho inhibitory stimuli on the blood flows in muscle and kidney both with intact vasomotor innervation
A Cortical stimulation (3 5 V 3 msec 30 imp /sec) at point (*1 19)
B and E Stimulation (0 5 V 2 msec 60 imp /sec) within the depressor area of the medullary vasomotor centre
C Rhytmic pulling on the occluded common carotid arteries
D Stimulation (4 V 2 msec 40 imp /sec) of the proximal end of the cut left vagus
The figure illustrates that the vascular responses in respective regions are essentially the same independent of the type of the sympatho inhibitory stimulus applied Renal blood flow changes are essentially passive due to the absence of any renal vasoconstrictor tone For further details see Fig 2

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Fig 18 illustrates an experiment of this type It is seen that a pronounced blood pressure fall is obtained on stimulation of the cingulate depressor area

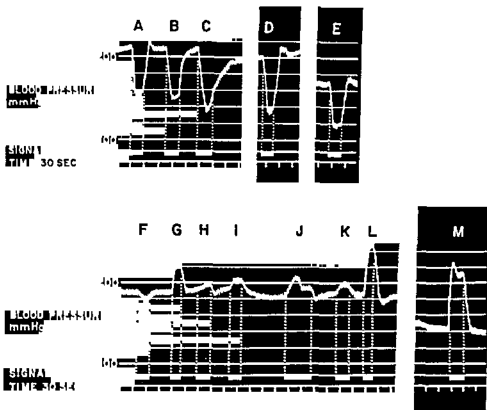


Fig 18 Cat 3 1 kg Chloralose Effects of different sympatho inhibitory stimuli on the arterial blood pressure before (A—E) and after (F—M) electrolytic lesion within the depressor area of the bulbar vasomotor centre

A and H Electrical stimulation (3 V, 3 msec, 25 imp /sec) of the cingulate depressor area

B, F and F Rhytmic pulling on the occluded common carotids

C, I and J Stimulation (4 V, 2 msec, 40 imp /sec) of the proximal end of the cut left vagal nerve

D and K Stimulation (0 7 V, 2 msec, 70 imp /sec) within the 'depressor area of the bulbar vasomotor centre, repeated in L with 3 V

G Electrical stimulation (4 V, 3 msec 60 imp /sec) of the cortical pressor area Between L and M both the carotid sinus nerves were blocked by local infiltration of a 2 per cent Xylocaine solution which caused the blood pressure to decrease

M Stimulation (4 V 2 msec 50 imp /sec) within the anterior hypothalamus to show that *the pressor pathways are still essentially intact, which is also obvious from G*

Note that the localized destruction of the medullary depressor area selectively eliminates the sympatho inhibitory effects, whether derived from the baroreceptors or from the cingulate depressor area For further details see Fig 2

(A) on electrical stimulation of the central end of the cut left vagal nerve (C) on topical stimulation of the medullary depressor area (D) as well as on excitation of the carotid baroreceptors caused by pulling on the occluded carotid arteries (B) repeated about 30 min later when the control blood pressure is somewhat decreased (E) After this sequence of stimulations the electrode placed within the medullary depressor area was used to produce an electrolytic lesion by applying a d c of about 10 mA for some min while the electrode position was shifted about 2 mm in the craniocaudal direction on both sides and also moved in the dorsoventral direction while the d c was applied After this procedure performed while the carotid occlusion was maintained the blood pressure is elevated some 20 mm Hg When it appeared as if the depressor area had been largely destroyed as judged by the virtual absence of any depressor response upon mechanical activation of the carotid baroreceptors the same series of stimulations are repeated Intense pulling on the occluded common carotids now results in only a minute blood pressure fall (F) far smaller than that obtained before the electrolytic lesion (B and E) indicating that the inhibitory effect of the baroreceptors on the tonic sympathetic activity is largely eliminated Stimulation within the cortical *pressor* area (G) produces a blood pressure increase and now the stimulation of the cingulate *depressor* area causes a slight pressor response (H) instead of the earlier marked depressor response (A) probably because even within this area some excitatory neurons are present Electrical stimulation of the left vagus together with the aortic depressor nerve results in a blood pressure rise (I and J) probably due to stimulation of the chemoreceptor fibres while the influence of the depressor fibres now appears to be centrally blocked by the electrolytic lesion of the medullary depressor area Topical stimulation of the depressor area of the medullary vasomotor centre (K) with the same

rise obviously due to a spread of the current to intact sympatho-excitatory fibres Local anesthesia of the sinus nerve produced by infiltration of the carotid sinus with 2 per cent Xylocaine induced a gradual blood pressure fall presumably due to the elimination of the carotid chemoreceptor excitatory influence The well maintained blood pressure and the considerable pressor response which can be induced by stimulation of the anterior hypothalamus (M) suggests that the medullary neurons responsible for tonic constrictor fibre activity are essentially unaffected by the electrolytic lesion of the medullary depressor area

It appears from this and the other experiments included in this series

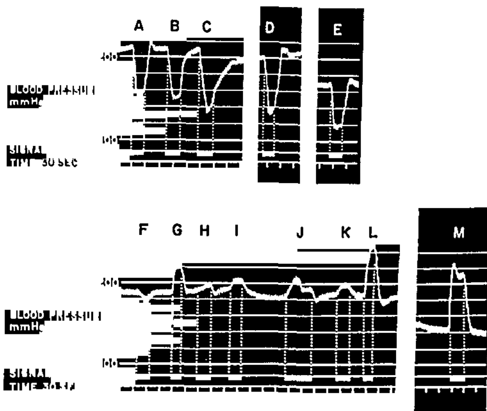


Fig 18 Cat 3 1 kg Chloralose Effects of different sympatho inhibitory stimuli on the arterial blood pressure before (A—E) and after (F—M) electrolytic lesion within the depressor area of the bulbar vasomotor centre

A and H Electrical stimulation (3 V 3 msec 25 imp /sec) of the cingulate depressor area

B E and F *Rhythmic pulling on the occluded common carotids*

C I and J Stimulation (4 V, 2 msec 40 imp /sec) of the proximal end of the cut left vagal nerve

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can be traced by the Marchi technique In all probability the fibres concerned with the autonomic responses belong to the small calibre fibre group, being either poorly myelinated or even unmyelinated If so, they can hardly be traced with the Marchi technique

WALL and DAVIS (1951) suggested that the autonomic fibres from the cingulate gyrus may bypass the hypothalamus, exerting their effects via the temporal lobes This suggestion was, however, based on only two observations in monkeys and the interest in this study was concentrated on the projections of the autonomic neurons from the motor and premotor area, the orbital surface of the frontal lobes and the temporal lobes

The present results, in some respects coinciding with those obtained in several earlier studies mentioned above, strongly suggest that the sympatho inhibitory effects, induced from the cingulate depressor area, are relayed via or pass through the hypothalamic sympatho inhibitory area described by FOLKOW, JOHANSSON and ÖBERG (1959) This structure may in fact form part of the ventromedial nucleus of the hypothalamus or of the fibre bundles reaching this nucleus from the cingulate depressor area

The results described in section (b) of the present chapter and illustrated in Fig 14—17, indicate that the pattern of regional vasoconstrictor fibre inhibition elicited from the cingulate depressor area, is not only qualitatively but even quantitatively similar to those induced from the cardiovascular baroreceptors and from the medullary 'depressor area' Further, the extent of the neurogenically induced regional vasodilation was always directly related to the prestimulatory level of vasoconstrictor fibre tone According to the results described in Chapter IV the constrictor fibres of the skeletal muscle vessels appear to be controlled by 'low threshold' neurons, exhibiting an especially intense tonic activity when released from reflex inhibitory influences Consequently, they can be especially markedly dilated when exposed to cortically or reflexly induced sympatho inhibitory influences, much more so than the other vascular beds studied, where the prevailing constrictor fibre activity was more moderate (intestinal vessels) small or even negligible (cutaneous and renal vessels) This also explains the, at first sight, somewhat puzzling lack of any neurogenically produced vasodilatation within e g the kidneys upon intense excitation of the different sets of sympatho inhibitory pathways studied As outlined in Chapter IV, the explanation seems to be that the neuron pools controlling the activity of the renal vasoconstrictor fibres can be considered to have an especially 'high threshold' with respect to the excitatory mechanisms responsible for the tonic activity of the bulbar vasomotor centre Hence they show no, or only negligible tonic discharge unless additional excitatory influences are superimposed If so,

as if the localized lesion of the medullary depressor area had fairly selectively eliminated both the cortically induced inhibitory effects on the tonic sympathetic activity as well as the strong inhibitory influences that can be elicited via the baroreceptor mechanisms Confirmatory examination of the damaged area under magnification indicated that it was confined to the midline structures with a bilateral extension of about 1 mm and a craniocaudal extension of about 2 mm in both directions from the medullary points where a topical stimulation induced the most extensive depressor response

Comments (*a*) (*b*) *and* (*c*) From the results obtained by topical stimulation within the cingulate depressor area before and after strictly localized lesions within the site of the hypothalamic sympatho inhibitory area it is reasonable to assume that the cortical fibres within the rostral cingulate gyrus exert their depressor influence via this hypothalamic structure This is further supported by the fact that the peripheral response patterns evoked from these cortical and hypothalamic depressor areas are virtually identical

It is relevant here to mention that KABAT MAGOUN and RANSON (1935) and HESS (1947) reported that blood pressure falls could be elicited by topical stimulations within a region extending from the rostral cingulate gyrus down to and including the septal preoptic and the hypothalamic areas These descending pathways thought to be closely linked to the ventromedial hypothalamic nucleus appear to coincide with those yielding inhibition of cortically and reflexly induced movements and also of the respiration (KAADA 1960 p 1359) It needs in this connection to be mentioned that destruction of the above hypothalamic nucleus in cats often altered their behaviour towards a more savage state (WHEATLEY 1944) This finding lends support to the view that this hypothalamic region may form an important relay station for more or less steady cortical inhibitory influence on lower integration centres as is suggested already by the well known findings of sympathetic release and sham rage in decorticated animals

However there are other studies which indicate that the centrifugal fibres from the rostral cingulate gyrus might take other routes Using a Marchi technique WARD (1948) found that the major fraction of the myelinated centrifugal pathways follows the internal capsule to reach ventromedial portion of the cerebral peduncles evidently without making connections with the hypothalamus It is quite possible however that the *efferent projections* of the highly differentiated cingulate gyrus may take several different routes depending on the functional engagement of the various sets of efferent fibres Those neurons mediating cardiovascular *and other autonomic effects* may take a quite different route from the thicker myelinated fibres which

To summarize the results described in Chapter V the cardiovascular sympatho inhibitory response pattern produced by activation of the cingulate depressor area appears to be relayed *via* the above mentioned hypothalamic sympatho inhibitory structures It is qualitatively and quantitatively closely similar or even identical to that obtained by activation of the cardiovascular baroreceptors or by topical stimulation of the medullary depressor area The magnitude of the regional vascular responses caused by activation of the different types of sympatho inhibitory mechanisms which appear to exert a generalized over all effect is closely correlated to the prestimulatory vasoconstrictor tone The ensuing vasodilatations thus become marked in tissues where constrictor fibre tone is intense moderate where it is of medium intensity and negligible where initially practically no constrictor fibre activity is present Electrolytic lesions of those parts of the medial bulbar reticular formation which constitute the so called depressor area of the vasomotor centre and which are known to mediate the inhibitory influence of the baro receptors abolish not only the inhibitory effects of these receptors on the tonic sympathetic activity but also those elicited from the cingulate depressor area Therefore the present findings strongly suggest that all these sympatho inhibitory mechanisms are mediated *via* the medullary depressor area

it is only natural that the different sets of sympatho inhibitory mechanisms studied could not produce any significant neurogenically mediated renal vasodilatation If however a renal vasoconstrictor fibre discharge was produced by the addition of an excitatory nociceptor fibre stimulation a marked inhibition of this renal vasoconstrictor tone could be produced by activation of the *cingulate depressor area* by *activation of the cardiovascular baro* receptors or by direct stimulation of the medullary depressor point The different types of sympatho inhibitory mechanisms also produced neurogenic vasodilatation of comparable size within the intestine and the skin Even here the extent of the inhibitory influence was closely correlated to the level of the prevailing constrictor fibre tone *These findings suggest when taken together that it is the tonic constrictor fibre discharge that is differentiated while the different sets of sympatho inhibitory pathways appear to exert a generalized inhibitory effect on this discharge wherever it is present*

So far as the fundamental organization of the nervous system underlying these observations is concerned it is tempting to postulate that the different sympatho inhibitory effects may be mediated *via* a common mechanism The findings discussed below would tend to support this concept

The results from the third series of experiments described in (c) of this chapter demonstrate how the sympatho inhibitory effects elicited from the cingulate depressor area as well as from the cardiovascular baroreceptors or from the medullary depressor area are markedly diminished or abolished after a topical electrolytic lesion of the medullary depressor area Although there exists no sharp borderline between the medullary pressor and depressor areas (OBERHOLZER 1960) it is possible to produce a functionally fairly selective *lesion of the depressor area as shown for example by* LINDGREN and UVNÄS (1954) In the present study the produced damage did not significantly affect pressor responses induced from the hypothalamic level or from different medullary points situated rostrally to the area of damage Moreover it converted the depressor effect induced by afferent vagal stimulation into a pressor effect presumably by allowing the chemoreceptor excitatory influence on the medullary pressor area to exert its influence unmasked by the inhibitory baroreceptor influence Nor did the medullary lesion eliminate the excitatory effects produced from the cortical pressor area demonstrating that the absence of the cortically induced depressor effect could hardly be *ascribed to any generalized depression* of cortical excitability The electrolytic damage is therefore assumed to be fairly selective with regard to the medullary structures which mediate the sympatho inhibitory influences which arise from both the cardiovascular baroreceptors and from the cingulate and hypothalamic depressor areas

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cortical stimulation or upon the exercise of reflex influences on the cardio vascular system

From an exploratory series of experiments outlined in *Chapter III* it was evident that sometimes very complex differentiated effects on the cardio vascular system mediated by the sympathetic adrenergic supply could be elicited from the limbic structures studied The latter obviously contain both sympatho inhibitory and excitatory neurons spatially so arranged as to form a relatively well defined depressor area around the genu of the corpus callosum and situated ventrally to this a pressor area Though the overlap between these inhibitory and excitatory neuron pools is relatively considerable topical stimulations within their central parts were able to produce clear cut sympatho inhibitory and excitatory response patterns respectively

As the analysis of the functional organization of the limbic sympatho inhibitory responses was the main purpose of the present study the sympatho excitatory responses that could be elicited from the pressor area were not more systematically investigated It was however evident from these exploratory experiments that this area was so organized as to be able to exert sometimes highly differentiated effects on lower sympathetic centres These preliminary results to be extended in subsequent studies suggest that the limbic sympatho excitatory neurons may exhibit a different extent of fibre convergence upon the autonomic neuron pools at lower levels of the nervous system these control the discharge of the constrictor fibres di tributed to the functionally different vascular regions

With regard to the exploration of the depressor area the preliminary ex periments suggested that this area too produced cardiovascular adju tments so organized as to provoke regional differences in the extent of inhibition of sympathetic tone This particular aspect is dealt with more extensively in Chapter IV and V For reasons discussed in detail in Chapter III the depressor responses were at best only moderate in extent as long as the po verful reflex adjustments induced by the cardiovascular baro and che moreceptors were still operating in the normal way Evidence wa however presented to indicate that at least under certain cond tions the sympatho inhil itory inf' ience exerted from the cingulate depressor area may be most dramatic In order to facilitate the analysis of the sympatho inhibitory responses elicited from the depressor area most experiments were therefore performed under circumstances where the modulating influence of the cardio vascular receptors was largely eliminated and thus the tonic sympathetic discharge released from its more important reflex inhibitory control These procedures partly compensated for the fact that the cortical structures were

CHAPTER VI

General conclusions and comments

In the present study an attempt has been made to analyse the functional organization of anterior parts of the limbic system which exert an inhibitory influence on the tonic activity of the adrenergic neurons participating in cardiovascular control As outlined in *Chapter I*, during recent decades a great number of studies has dealt with the organization and functional significance of the highly complex limbic system However, comparatively rarely have these been devoted to its influence on the cardiovascular system and few, *if any*, attempts have so far been made to study in more detail the way in which these cortical structures affect the circulation Previous investigations can therefore mainly be looked upon as exploratory but in this respect *they have been of great importance Thus, among other things they indicate* that circulatory reactions may be elicited from the anterior parts of the cingulate gyrus and adjacent sections of the subcallosal gyrus These may be of considerable significance in the control of the circulation, the present study has, therefore, been devoted to investigating these anterior parts of the limbic system

When the present investigation was commenced, initial experiments clearly demonstrated that a more detailed analysis of the influence of such cortical structures on sympathetic adrenergic fibre discharge required relatively elaborate technical procedures if the autonomic patterns, affecting the cardiovascular system, were to be understood Therefore as was described in *Chapter II*, the experiments were so devised as to exclude as far possible other variables that might affect the cardiovascular system It was necessary to record several of the more important parameters in one and *the same experiment in order* to reveal possible regional variations in constrictor fibre adjustments to different vascular beds Since electrophysiological methods implying direct recordings of fibre activity *were considered to be less suitable for this special* purpose (see Chapter II), the effector responses in representative cardiovascular sections were concomitantly recorded instead With the availability of data relating the average discharge rate in vasomotor fibres and the ensuing effector response within the vascular regions studied it proved possible to evaluate the average changes in constrictor fibre discharge taking place upon

cortical stimulation or upon the exercise of reflex influences on the cardio vascular system

From an exploratory series of experiments outlined in *Chapter III* it was evident that sometimes very complex differentiated effects on the cardio vascular system mediated by the sympathetic adrenergic supply could be elicited from the limbic structures studied The latter obviously contain both sympatho inhibitory and excitatory neurons spatially so arranged as to form a relatively well defined depressor area around the genu of the corpus callosum and situated ventrally to this a pressor area Though the overlap between these inhibitory and excitatory neuron pools is relatively considerable topical stimulations within their central parts were able to produce clear cut sympatho inhibitory and excitatory response patterns respectively

As the analysis of the functional organization of the limbic sympatho inhibitory responses was the main purpose of the present study the sym patho excitatory responses that could be elicited from the pressor area were not more systematically investigated It was however evident from these exploratory experiments that this area was so organized as to be able to exert sometimes highly differentiated effects on lower sympathetic centres These preliminary results to be extended in subsequent studies suggest that the limbic sympatho excitatory neurons may exhibit a different extent of fibre convergence upon the autonomic neuron pools at lower levels of the nervous system these control the discharge of the constrictor fibres distributed to the functionally different vascular regions

With regard to the exploration of the depressor area the preliminary ex periments suggested that this area too produced cardiovascular adjustments so organized as to provoke regional differences in the extent of inhibition of sympathetic tone This particular aspect is dealt with more extensively in Chapter IV and V For reasons discussed in detail in Chapter III the depressor responses were at best only moderate in extent as long as the powerful reflex adjustments induced by the cardiovascular baro and che moreceptors were still operating in the normal way Evidence was however presented to indicate that at least under certain conditions the sympatho inhibitory influence exerted from the cingulate depressor area may be most dramatic In order to facilitate the analysis of the sympatho inhibitory responses elicited from the depressor area most experiments were therefore performed under circumstances where the modulating influence of the cardio vascular receptors was largely eliminated and thus the tonic sympathetic discharge released from its more important reflex inhibitory control These procedures partly compensated for the fact that the cortical structures were

inevitably much depressed by the necessary application of general anesthesia and put them in a more favourable competitive position towards the counter *acting buffer reflexes*

It was regularly found that considerable depressor responses could be elicited when the appropriate part of the limbic cortex was stimulated at frequencies of only a few impulses per second and that almost maximal depressor effects were reached at stimulation frequencies of 25—30 impulses sec At higher frequencies the depressor response was *often established more* rapidly but on the other hand, it tended to vanish in spite of a continued high frequency stimulation A shift to lower frequency re established the depressor response These observations indicate that normally the e fibres may fire at very low rates presumably well below 20—30 impulses ec

The results of a series of preliminary experiments on conscious animals was reported where the effects of topical stimulation of the limbic depressor area were observed with respect to changes of general behaviour respiration and *skeletal muscle activity These preliminary observations suggest that normally* the sympatho inhibitory pattern may be combined with a generalized inhibition of the somatomotor system depressing for instance normal withdrawal responses respiration etc

In Chapter IV the depressor responses elicited from anterior parts of the limbic system were analysed in more detail in order to reveal the background of the obvious regional differences in extent of the sympatho inhibitory effects on the cardiovascular system It was found that topical excitation *of the cingulate depressor area — besides inducing a sometimes considerable* activation of the vagal heart fibres — was also able to cause a profound inhibition of the prevailing tonic activity of the sympathetic accelerans and vasoconstrictor fibres while the cholinergic sympathetic vasodilator fibres were not engaged Nevertheless the ensuing vasodilatation was always most pronounced within the skeletal muscles to such an extent indeed that muscle blood flow generally increased in spite of often drastic reductions of perfusion pressure The vessels of the intestines were less *markedly affected while only small or even insignificant decreases of flow resistance* were seen within the skin and the kidneys In estimating the regional decreases of discharge rate of vasoconstrictor fibres these findings strongly suggest that the constrictor fibre activity was markedly decreased within the muscles *less so within the* intestines and very little or sometimes not at all within the skin and the kidneys A possible contribution of shifts in the hormone discharge from the adrenal medullae could be excluded both because elimination of this hormone release did not affect the response pattern induced by cortical stimulation and because the vascular effects were entirely eliminated when the regional

vasoconstrictor fibres were cut A possible criticism of this interpretation is that regional differences in extent of vasodilatation may not be due to actual differences in the extent of fibre discharge changes but rather reflect an uneven constrictor fibre distribution and/or regional differences in effector sensitivity to the adrenergic transmitter If for instance the muscle vessels had the most abundant constrictor fibre supply they would be relatively more constricted by a uniform increase of tonic discharge than the vessels of other tissues and — conversely — be more strongly dilated at a uniform decrease of constrictor fibre activity If this was so it would not be necessary to assume any differentiation either of the tonic constrictor fibre discharge or of the cortical inhibitory influences depressing this tonic activity marked regional differences in vascular responses to neurogenic adjustments would nevertheless ensue

Considerable regional differences in fibre distribution do occur and presumably there are also differences in effector cell responsiveness to a given amount of the adrenergic transmitter (see *e g* FOLKOW 1955 1960) However these factors cannot explain the present findings simply because known data concerning regional differences in vasoconstrictor fibre effects make it clear that a quite different pattern to that observed in the present experiments would have been induced if a uniform shift in constrictor fibre discharge rate had occurred Thus data presented by CELANDER and FOLKOW (1953) and by CELANDER (1954) and repeatedly confirmed in the present experiments have shown that the cutaneous vessels especially those of the paws are much more strongly affected by a given discharge rate than are muscle vessels In addition at one and the same constrictor fibre discharge the renal vessels are almost as strongly affected as are the muscle vessels and this is even the case with the intestinal vessels (CELANDER unpublished observations) Therefore the neurogenically induced flow changes occurring as a response to stimulation of both the cingulate pressor and depressor areas are incompatible with the view that the constrictor fibre discharge should be equally affected within the different vascular circuits The fact that hypothalamic stimulations or afferent stimulations of the sciatic nerve were able to produce extensive renal vasoconstrictions — phenomena that will be discussed in more detail in subsequent publications (JOHANSSON 1961, LOFVING 1961) — makes it clear that the above mentioned almost complete absence of renal vascular effects was not due to any damage inflicted upon the renal constrictor fibre pathways Thus it is necessary to assume that regional differences in constrictor fibre discharge must have occurred and the question then arises as to how these differences are brought about

The regional differences in the decreases of constrictor fibre activity and

hence in vascular response could in principle be due either to a cortically induced differentiated inhibition of an equally distributed vasoconstrictor tone or be the result of a more generalized inhibitory influence on a prevailing difference in the distribution of nervous vasoconstrictor tone The second alternative would imply that the tonic discharge of the vasomotor centre when released from the damping baroreceptor influence and or excited by the chemoreceptors was differentiated in such a way that the muscle vessels under the existing experimental conditions were exposed to a higher constrictor fibre discharge rate than were *e g* the renal vessels A diffuse overall inhibitory influence would then be expected to induce a more pronounced vasodilatation within those tissues where the nervous vasoconstrictor tone was initially high while no significant effects could be expected in tissues where the neurogenically induced vascular tone was negligible Of course both these alternatives could be of actual importance insofar as the tonic constrictor fibre discharge as well as the cortically induced inhibitory effect might be differentiated to some extent

However according to the results obtained in the series of experiments reported in the second part of Chapter IV, it seems clear that the specific pattern of vasodilator responses elicited from the cingulate depressor area is basically a matter of differentiation of the constrictor fibre tone The tonic discharge of the VMC when released from the baroreceptor inhibition and/or excited by the chemoreceptors is especially marked in the fibres supplying the muscle vessels moderate in those supplying the intestinal vessels and generally weak or almost absent in those controlling the cutaneous and the renal vessels (LÖFVING 1961) It appears in fact that the autonomic neuron pools controlling the functionally different vascular beds exhibit somewhat different thresholds with regard to excitatory influences The medullary neuron pools controlling *e g* the renal vessels can be considered to be high threshold in this respect showing little or no tonic activity in circumstances when they are not concomitantly exposed to additional excitatory influences emanating from excitatory hypothalamic structures nociceptor *fibres or* simply caused by exposing the animal to severe asphyxia (FOLKOW JOHANSSON and LÖFVING 1961) Such procedures can bring about a considerable discharge of the renal vasoconstrictor fibres and it can then be shown as reported in Chapter V that stimulation of the cingulate depressor area exerts a sympatho inhibitory influence on the renal vascular bed also These data suggest that it is the tonic constrictor fibre activity *that is differentiated a* phenomenon based on the simple principle of quantitative differences in excitability level of the different medullary neuron pools while the limbic sympatho inhibitory influence appears *to be generalized and diffuse*

In *Chapter I* some aspects of the efferent pathways from the cingulate depressor area are discussed especially concerning their relationship to the hypothalamic sympatho inhibitory centre (FOLKOW JOHANSSON and ÖBERG 1959) and to the so called depressor area of the bulbar vasomotor centre In addition the cortically induced sympatho inhibitory pattern was quantitatively compared with those induced by topical stimulation within the medullary depressor area and by activation of different types of cardiovascular baroreceptors Briefly the results suggest that the cingulate sympatho inhibitory neurons pass via or are relayed in the above mentioned hypothalamic sympatho inhibitory structure and the part of the medial bulbar reticular formation called the medullary depressor area In addition it was found that the cortically induced sympatho inhibitory pattern was as far as could be judged identical in nature with those induced via the cardiovascular baroreceptors In fact both the reflexly and the centrally induced sympatho inhibitory responses appear to have a common structural denominator insofar as they all seem to be relayed via the depressor area of the medial bulbar reticular formation So it may well be that most if not all sympatho inhibitory effects — whether induced from central parts of the nervous system or via peripheral receptors — exert their inhibitory influence on tonic sympathetic activity by way of one and the same part of the reticular formation at the medullary level

To summarize the centrally induced sympatho inhibitory responses appear to be combined with an activation of the vagal heart fibres and with an inhibition of respiration and with somatomotor control in general It should here be especially stressed that the sympathetic cholinergic vasodilator fibres are not engaged in this pattern of response Recent experiments (ABRAHAMS HILTON and ZBROZYNA 1960) have presented strong evidence to show that these dilator fibres form an important part of an almost contrary directed response pattern the so called alarm reaction implying generally a vigorous activation both of the cardiovascular system and the somatomotor system

The question then arises as to the possible functional significance of the studied sympatho inhibitory pattern and its probable somatomotor inhibitory correlate Before this question is discussed it should be realized that great precautions must be taken when response patterns induced by topical stimulations of the cortex are considered Even strictly localized topical stimulations are of necessity a crude mode of activation of higher structures because the elicited response patterns are probably not an exact reproduction of a physiologically induced one Due to possible structural overlaps it is difficult to exclude the possibility that the induced responses are either fragmentary or

hence in vascular response could in principle be due either to a cortically induced differentiated inhibition of an equally distributed vasoconstrictor tone or be the result of a more generalized inhibitory influence on a prevailing difference in the distribution of nervous vasoconstrictor tone. The second alternative would imply that the tonic discharge of the vasomotor centre when released from the damping baroreceptor influence and/or excited by the chemoreceptors was differentiated in such a way that the muscle vessels under the existing experimental conditions were exposed to a higher constrictor fibre discharge rate than were e.g. the renal vessels. A diffuse overall inhibitory influence would then be expected to induce a more pronounced vasodilatation within those tissues where the nervous vasoconstrictor tone was initially high while no significant effects could be expected in tissues where the neurogenically induced vascular tone was negligible. Of course both these alternatives could be of actual importance insofar as the tonic constrictor fibre discharge as well as the cortically induced inhibitory effect might be differentiated to some extent.

However according to the results obtained in the series of experiments reported in the second part of Chapter IV it seems clear that the specific pattern of vasodilator responses elicited from the cingulate depressor area is basically a matter of differentiation of the constrictor fibre tone. The tonic discharge of the VMC when released from the baroreceptor inhibition and/or excited by the chemoreceptors is especially marked in the fibres supplying the muscle vessels moderate in those supplying the intestinal vessels and generally weak or almost absent in those controlling the cutaneous and the renal vessels (LOFVING 1961). It appears in fact that the autonomic neuron pools controlling the functionally different vascular beds exhibit somewhat different thresholds with regard to excitatory influences. The medullary neuron pools controlling e.g. the renal vessels can be considered to be high threshold in this respect showing little or no tonic activity in circumstances when they are not concomitantly exposed to additional excitatory influences emanating from excitatory hypothalamic structures nociceptor fibres or simply caused by exposing the animal to severe asphyxia (FOLKOW JOHANSSON and LOFVING 1961). Such procedures can bring about a considerable discharge of the renal vasoconstrictor fibres and it can then be shown as reported in Chapter V that stimulation of the cingulate depressor area exerts a sympatho inhibitory influence on the renal vascular bed also. These data suggest that it is the tonic constrictor fibre activity that is differentiated a phenomenon based on the simple principle of quantitative differences in excitability level of the different medullary neuron pools while the limbic sympatho inhibitory influence appears to be generalized and diffuse

pressure fall and may sometimes even exceed the flow in a sympathectomized muscle region — appears indeed to be a good indirect support for an engagement of a specific dilator mechanism However, it is quite often observed in animal experiments that on sudden elimination of a tonic constrictor fibre influence on the muscle vessels, a brief 'overshoot' in flow occurs, beyond the level of flow seen a few minutes after an acute sympathectomy A reasonable explanation of such an overshoot in flow is that the muscle vessels exhibit a type of 'reactive hyperaemia' when they are suddenly released from the restricting influence of the constrictor fibres Further, the present experiments have uniformly shown that the constrictor fibre inhibition especially strongly and promptly affects the muscle vessels It is, hence possible that also the muscle blood flow changes seen in man on emotional fainting can be explained as being due to a pure constrictor fibre inhibition In addition the fact that the vasodilator fibres appear to be engaged in a quite different activation pattern the defense or alarm reaction with its intense sympathetic activation (BLAIR *et al* 1959, ABRAHAMS, HILTON and ZBROZYNA 1960), makes it in a way less likely that these specialized vasomotor fibres should also take part in a generalized sympatho inhibitory response, which in many respects is in complete contrast to the defence reaction

Therefore on the basis of the present observations and the above mentioned considerations it is suggested as a working hypothesis that the cingulate sympatho inhibitory area forms one of the cortical structures that appear normally to exert a more or less steady damping effect on lower sympathetic centres including those controlling the cardiovascular system It appears, however that this cingulate depressor area does not exclusively engage the autonomic nervous system it can in addition exert a generalized inhibitory influence on respiration and on somatomotor control thereby forming one of the central conveyors of the somato and visceromotor expressions accompanying changes in alertness and in emotional balance In certain situations this inhibitory influence might become so strongly intensified that very extensive generalized inhibitions occur Accordingly, it may be responsible for such cortically induced, drastic responses as the 'playing dead' reaction of some animal species and its possible human correlate, emotional fainting If so these cortical inhibitory structures may be functionally as important and interesting as those responsible for the defence alarm reaction, which, due to its extensive visceromotor and somatomotor excitation, appears to form the extreme opposite to the inhibitory response pattern outlined in the present study

mixtures of different physiological patterns Many additional studies are therefore needed before the working hypothesis outlined below can be looked upon as firmly established With such reservations in mind it is nevertheless of interest even at the present stage of knowledge, to discuss the functional significance of the findings obtained in this study

There are many experimental observations suggesting that parts of the cerebral cortex normally exert a more or less steady inhibitory influence on *general behaviour, emotional expressions and on lower sympathetic centres* in general The well known phenomena of sham rage, accompanied by violent sympathetic outbursts on even trifling provocations in decerebrate animals indicate that normally diencephalic and other lower integrative centres are subject to more or less steadily operating cortical inhibitory influences Several cortical inhibitory systems may here be engaged but it is reasonable to assume that the limbic structures presently studied might be of considerable importance the more so as other studies (see Chapter I) suggest that the limbic system is engaged in emotional behaviour and expressions It should also be appreciated that *e g* the cardiovascular adjustments seen in changes of alertness or of emotional balance are not necessarily mainly a matter of an engagement of cortical sympatho excitatory structures It may well be that modulations of a more or less steadily operating sympatho inhibitory cortical influence are even more important It is therefore not impossible that the studied topically excited sympatho inhibitory structures are in fact continuously operating to a moderate extent in the intact organism and affected in both directions by shifts in alertness and emotional balance depending on the nature of stimuli imposed on the cortex

Some more pronounced inhibitory reactions induced in certain mental stress situations may be manifestations of a suddenly exaggerated activity of structures like the limbic sympatho inhibitory area It is tempting to speculate whether phenomena like the playing dead reactions of some animal species or the emotional fainting in man — being in some respects the extreme *opposite to the alarm defence reaction* — may be the result of sudden intense activation of these cortical inhibitory structures No doubt the pattern of cardiovascular shifts taking place in emotional fainting in man like that *induced by a drastic baroreceptor activation* are very similar and possibly identical with the cortically induced depressor responses studied here In all these reactions there are signs of a vagal bradycardia and a generalized inhibition of *constrictor fibre tone It has however* earlier been suggested that the sympathetic vasodilator fibres might be activated also in emotional fainting in man (BARCROFT and EDHOLM 1945) *The evidence taken to support* this view — *the fact that muscle blood flow increases in spite of the blood*

pressure fall and may sometimes even exceed the flow in a sympathectomized muscle region — appears indeed to be a good indirect support for an engagement of a specific dilator mechanism However it is quite often observed in animal experiments that on sudden elimination of a tonic constrictor fibre influence on the muscle vessels a brief overshoot in flow occurs beyond the level of flow seen a few minutes after an acute sympathectomy A reasonable explanation of such an overshoot in flow is that the muscle vessels exhibit a type of reactive hyperaemia when they are suddenly released from the restricting influence of the constrictor fibres Further the present experiments have uniformly shown that the constrictor fibre inhibition especially strongly and promptly affects the muscle vessels It is hence possible that also the muscle blood flow changes seen in man on emotional fainting can be explained as being due to a pure constrictor fibre inhibition In addition the fact that the vasodilator fibres appear to be engaged in a quite different activation pattern the defense or alarm reaction with its intense sympathetic activation (Blair *et al* 1959 Abrahams Hilton and Zbrozyna 1960) makes it in a way less likely that these specialized vasomotor fibres should also take part in a generalized sympatho inhibitory response which in many respects is in complete contrast to the defence reaction

Therefore on the basis of the present observations and the above mentioned considerations it is suggested as a working hypothesis that the cingulate sympatho inhibitory area forms one of the cortical structures that appear normally to exert a more or less steady damping effect on lower sympathetic centres including those controlling the cardiovascular system It appears however that this cingulate depressor area does not exclusively engage the autonomic nervous system it can in addition exert a generalized inhibitory influence on respiration and on somatomotor control thereby forming one of the central conveyors of the somato and visceromotor expressions accompanying changes in alertness and in emotional balance In certain situations this inhibitory influence might become so strongly intensified that very extensive generalized inhibitions occur Accordingly it may be responsible for such cortically induced drastic responses as the playing dead reaction of some animal species and its possible human correlate emotional fainting If so these cortical inhibitory structures may be functionally as important and interesting as those responsible for the defence alarm reaction which due to its extensive visceromotor and somatomotor excitation appears to form the extreme opposite to the inhibitory response pattern outlined in the present study

General summary

The purpose of the present study has been to elucidate in more detail the functional organization of the neuron pools within rostral parts of the limbic system, notably the anterior cingulate gyrus and the subcallosal region known to affect the cardiovascular system After an initial series of exploratory experiments interest was focussed on the cortically elicited inhibitory effects on lower sympathetic centres controlling the tonic activity of the adrenergic cardiovascular fibres

The exploratory experiments indicated that both 'sympatho excitatory and 'sympatho inhibitory' neurons exist within the mentioned parts of the cerebral cortex Though these two types of neuron pools appear to be inter mingled to some extent, they nevertheless form relatively well delinated 'pressor and 'depressor' areas When the powerful buffering effect of the cardiovascular baroreceptors was largely eliminated so as to reveal the full extent of the cortically induced circulatory changes, it was observed that often very pronounced sympatho inhibitory effects could be induced from the cortical depressor area This is situated within rostral parts of the cingulate gyrus sur rounding the genu of the corpus callosum Sympatho excitatory effects of often considerable magnitude could be induced from the pressor area situated within the subcallosal region a few mm ventrally to the cingulate depressor area

Observations of the changes in arterial blood pressure regional blood flow and on heart rate revealed that from closely situated cortical structures within the subcallosal pressor area sometimes fairly selective adjustments of either muscle renal or intestinal vessels could be induced suggesting a potentiality for differentiated excitatory influences on the constrictor fibres supplying the functionally different vascular beds The cutaneous vessels were relatively little engaged but were markedly affected when cortical structures situated still more ventrally were stimulated

In contrast stimulation of different points within the cingulate depressor area showed a remarkable constancy in the ensuing pattern of neurogenic cardiovascular adjustments Besides an activation of vagal heart fibres an inhibition of the tonic activity of the adrenergic sympathetic fibres to the heart and the systemic blood vessels could be induced from this region There was no evidence of any engagement of the sympathetic cholinergic vasodilator fibres distributed to the skeletal muscles Nevertheless the vaso dilatation caused by stimulation of the cingulate depressor area was almost always most pronounced within the skeletal muscles It was generally only moderate within the intestines and small within the skin while the renal vessels usually remained almost unaffected

Evidence was presented to show that the often striking regional differences in the vascular responses induced from the cortical depressor area must be ascribed to a differentiation of the prevailing vasoconstrictor fibre discharge Thus if the tonic activity of the medullary vasomotor centre was released from the inhibitory influence of the baroreceptors and/or excited by the chemo receptors the fibres to the skeletal muscles were especially strongly engaged those supplying the intestinal vessels more moderately so and those supplying the cutaneous and the renal vessels hardly at all Consequently the extent of the vasodilator responses caused by activation of the cingulate depressor area will be especially pronounced within the skeletal muscles moderate within the intestines and small or negligible within the skin and the kidneys in spite of the fact that the cortical sympatho inhibitory neurons appear to exert a generalized inhibitory effect

The sympatho inhibitory fibres of the cingulate depressor area appear to pass via the sympatho inhibitory area in the anterior hypothalamus An electrolytic lesion of this structure eliminated fairly selectively the cortically induced depressor effects

The pattern of neurogenic cardiovascular adjustments induced by stimulation of the cingulate depressor area was found to be qualitatively and quantitatively similar to those obtained upon topical stimulation of the medullary depressor area and upon activation of the cardiovascular baroreceptors Electrolytic lesion of the medullary depressor area abolished both the cortically induced depressor effect and those induced by baroreceptor activation without significantly affecting the pressor responses induced from the subcallosal pressor area These findings suggest that all these sympatho inhibitory mechanisms whether emanating from cortical structures or from the baroreceptors exert their cardiovascular effects via the medial part of the bulbar reticular formation which constitutes the depressor area of the vasomotor centre

The rostral cingulate gyrus forms an important part of those limbic structures supposed to be of fundamental importance for emotional experience and expressions It is also known that parts of the cerebral cortex normally exert inhibitory influences on certain lower autonomic and somatomotor integration centres Preliminary experiments in conscious animals suggest that the cortically induced generalized sympatho inhibitory response and the activation of the vagal heart fibres may be combined with a generalized inhibition of spontaneous somatomotor activity as well as with a temporary depression of the respiration

As a working hypothesis it is therefore suggested that the inhibitory neuron pools within the anterior cingulate gyrus form part of the cortical system

which normally affects certain lower somato- and visceromotor integration centres. It may exercise a more or less steady inhibitory influence varying in extent according to the actual state of alertness and emotional equilibrium.

The possibility is also discussed whether especially intense activations of such inhibitory cortical structures, a pattern which appear to be the functional opposite of those integrating the alarm defence reaction, may be responsible of phenomena like emotional fainting in man and the "playing dead" reactions occurring in some animal species.

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AND THE DEPARTMENT OF EXPERIMENTAL SURGERY,
KAROLINSKA SJUKHUSET, STOCKHOLM, SWEDEN

STUDIES ON CATECHOLAMINE-CONTAINING GRANULES OF SPECIFIC CELLS IN CYCLOSTOME HEARTS

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CONTENTS

STOCKHOLM 1961
KUNGL. BOKTRYCKERIET P. A. NORSTEDT & SÖNER

It has been previously reported that the heart of the cyclostome *Myxine glutinosa* (hagfish) contains remarkably high amounts of adrenaline and noradrenaline (Östlund 1954). This was confirmed and extended in further studies by Augustinsson *et al.* (1956) and by Östlund *et al.* (1960). The amounts of adrenaline in the atrium of another cyclostome *Petromyzon fluviatilis* (syn. *Lampetra fluviatilis*) (lamprey) were found to be higher than those in any other organ reported (118—140 μg/g) except for chromaffin cell tissue. It was also found that while noradrenaline was the predominating amine in the atrium of *Myxine*, adrenaline occurred in a much higher proportion in the ventricle. Since it was considered unlikely that the high amounts of catecholamines should be present in adrenergic nerves, the hearts were investigated for the presence of possible storage cells. Thus Östlund *et al.* (1960) presented evidence of a specific granular cell in the hearts of the cyclostomes *Myxine* and *Petromyzon*. These cells contained granules which were chromaffin and osmiophilic, suggesting that they were involved in storage and release of the catecholamines and possibly related to those granules found in the chromaffin cells of the adrenal medulla (Blaschko and Welch 1953, Hillarp, Lagerstedt and Nilson 1953).

Preliminary experiments had also shown that incubation of isolated granules from the hearts of *Petromyzon* and *Myxine* with reserpine caused an increased rate of release of the catecholamines.

In the present paper the specific cells and their storage granules have been further studied with histological and electron microscopical techniques. The distribution and occurrence of catecholamines and other catechol substances in extracts of cyclostome hearts has been studied with chromatographic technique. The effects of reserpine on the storage granules *in vivo* and *in vitro* are also described. In addition, some findings concerning the action of catecholamines on the activity of the isolated heart from *Myxine* after pretreatment with reserpine are reported.

cutaneous injection of doses of 0 1—0 2 mg to specimens maintained in tap water (lamprey) or sea water (hagfish) in the laboratory The injections were repeated daily during periods of up to 7 days

Chromatography Chromatographic separation of the catechols in the cyclostome hearts was made in a number of cases after adsorption of trichloroacetic acid extracts on alumina and elution with 0 25 N sulphuric acid After concentration in vacuo the eluate was saturated with NaCl and the catechols taken up in isobutanol The butanol extracts were subjected to chromatography on a starch column, using a solvent of n butanol, 0 1 N hydrochloric acid and 10 N acetic acid (5 1 2) Aliquots of the different fractions (2 2 ml) were treated with ethylene diamine and the fluorescence of the condensation product measured in a Coleman 12 C photofluorimeter

Assay of histamine and 5-HT An extract of *Petromyzon* heart was made with 5 per cent trichloroacetic acid which was subsequently removed with ether After inactivation of the catecholamines with MnO_2 or oxygen at pH 7 0 the extract was assayed for histamine on the isolated guinea pig ileum Another group of hearts were extracted with 1 N HAc and the extract was passed over a column of amberlite XE 64 in H^+ form Elution was performed with 3 + 3 ml 1 2 N HCl, and after addition of HCl to 3 N the solution was examined in the Aminco spectrophotofluorimeter for the presence of 5 HT

Heart catecholamine granules Catecholamine containing microgranules were prepared by the technique described by EULER and LISHAJKO (1961 b) and, either directly or after

[illegible]

added to the perfusion fluids which were fed to the heart separately and under constant pressure

MATERIAL AND METHODS

Light microscopy Hearts from *Myxine glutinosa* and *Petromyzon fluviatilis* were removed immediately after decapitation of the fishes For various staining procedures heart specimens were fixed in Bouin's and Bodian's fluids, ethanol formaldehyde (9 1), neutral formalin, Champy-Coujard's (CHAMPY and HATEM 1955) and Helly's fluids. In order to study chromaffinity, Orth's solution and the 5 % potassium bichromate-chromate mixture of HILLARP and HOKFELT (1955) were used The hearts were first perfused with the fixatives and then placed in the fixing solutions for various periods of time To obtain a standard for chromaffinity, fresh adrenal glands from rats, guinea pigs and human fetuses were similarly fixed and treated

Hearts and adrenal controls, fixed in chrome containing solutions, were frozen sectioned or embedded in polyethylene glycol or in paraffin Untreated mounted sections were then investigated in the light microscope for a chromaffin reaction

Specimens fixed in other fixation fluids were dehydrated and embedded in paraffin in the usual manner From the tissue blocks 5 μ sections were prepared except for the silver impregnations, which demand thicker sections (15 μ)

Fresh frozen specimen sections which had been floated out onto a formol calcium chloride solution according to ERANKO (1956) were investigated for fluorescence in a Zeiss Grosse Fluoreszenzeinrichtung" equipped with a mercury high pressure lamp and Schott-filters BG 12, OG 4 and OG 5

Other sectioned specimens were treated according to one or several of the following methods A haematoxylin eosin stain was used for general overall pictures Silver im- [illegible] according to a modified Palmgren method (PALMGREN 1960), [illegible] ilver method [illegible] the Schmorl [illegible] s of reducing substances Lastly, the chrome haematoxylin method of Gomori as modified by BARGMANN (1950) for neurosecretory substances was carried out

Electron microscopy Specimens from hearts of 8 lamprey and 6 hagfish were investigated by electron microscopy The fishes were decapitated and the hearts removed immediately In all cases the hearts were still beating when isolated Specimens from atrium and ventricle of both species and also from the portal vein heart of hagfish were rapidly fixed in veronal-buffered 1—2 % osmium tetroxide (PALADE 1952) They were then dehydrated and embedded in butyl methacrylate with benzoyl peroxide as a catalyst (NEWMAN, BORYSKO and SWERDLOW 1949) or in the epoxy resin EPON 812[1], according to the method described by FINCK (1960) Sectioning of the tissue blocks was performed on a Leitz ultramicrotome using glass knives Sections were examined in an RCA EMU 2 b electron microscope at original magnifications of 2,000—15,000 ×

Catecholamine assay Hearts were isolated and extracted with 5 per cent trichloroacetic acid, the extracts adsorbed on alumina and eluted with 0 25 N acetic acid The eluate was analyzed for adrenaline and noradrenaline according to a fluorimetric technique (EULER and LISHAJKO 1961 a) Reserpine (Serpasil®) was administered by sub-

[1] Shell Chemical Corp , Sewaren, New Jersey

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[1] Shell Chemical Corp , Sewaren, New Jersey



Fig 1 Light photomicrograph of *Petromyzon* atrium showing the widely dispersed system of dark staining specific cells Modified Palmgren method Magnification around 150 ×



anastomose quite as much as those of *Myxine* With regard to distribution within the lamprey heart though the cells are widely spread there is generally a much greater accumulation of them in the atrium especially in that part which surrounds the atrio-ventricular junction

As silver stains have been widely used in the present investigation special attention was paid to the possible occurrence of nerve fibres within the cyclostome hearts While fine nerve fibres were occasionally observed in the hearts of lamprey no similar structures were seen in hagfish Among the specific cells

RESULTS

I. *Morphological studies*

Anatomy The *Myxine* heart is enclosed in a large, thin-walled sac and consists of a sinus venosus, atrium and ventricle This species also has a well developed portal vein heart The pericardial cavity has an open communication with the abdominal cavity (FAVARO 1908, PIETSCHMANN 1934) The heart of *Petromyzon* differs somewhat from the given description Besides being enclosed by a cartilaginous pericardium a portal vein heart is lacking in this species

Histology and histochemistry

The contractile elements of the cyclostome hearts are the striated muscle cells which in the light microscope do not differ in appearance from those of higher animals The heart muscle tissue is rather loosely woven, resulting in a somewhat spongy structure with numerous blood-filled compartments This arrangement is probably necessary as these fishes lack a coronary circulatory system

A thorough histological investigation of the hearts of *Petromyzon* and *Myxine* has revealed the presence of a large number of specific granular cells spread throughout the heart muscle tissue forming a cellular system of impressive art (Fig 1)

In *Myxine glutinosa* light microscopical examination shows the specific cells to be evenly distributed throughout the whole heart (atrium, ventricle and portal vein heart) The cells are often located on the surface of the heart muscle bundles and thus generally lie just beneath the endocardium The cells vary somewhat in size (8—15 μ) and show a large nucleus with a loose chromatin network and often one or two prominent nucleoli The shape of the cells shows wide variations Generally they are long and spindle-shaped (Fig 2a) but they can also be rather broad and highly branched (Fig 2b) Protoplasmic protrusions are almost a rule and these sometimes reach considerable lengths Certain protrusions have been followed for 60—70 μ They protrude from the cell body and extend in between the surrounding muscle cells While the cellular projections are often seen to end in thin processes, which appear to be in intimate contact with neighbouring muscle fibres, it is not uncommon to see them anastomose with similar processes from other specific cells This often gives a 'syncytical'' appearance to the cellular system

In the other species investigated, *Petromyzon fluviatilis*, specific cells, very similar to those described in *Myxine* are found Their general appearance and staining reactions correspond well to those in the hagfish Their size, however, seems to vary somewhat more (6—18 μ) and they do not seem to branch and



F g 1 Light photom crograph of *Petromy on* atrium showing the widely dispersed system of dark staining spec fic cells Mod fied Palmgren method Magn ficat on around 150 ×



F g 2 Light m croscop cal p ch

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of *Petromyzon* there are also some elements whose appearance does not preclude the possibility that they might be *ganglion cells*

The *specific cells* are not easily visualized after ordinary fixatives followed by general stains such as haematoxylin and eosin However, *silver impregnation* methods, if properly applied, will bring out the *cells, as is the* case with a modified Palmgren stain in which the specific cells appear *in a colour* tone *that varies from* reddish-brown to bluish-black Upon closer examination it is seen that the cytoplasm has a fine granular appearance and that the *minute* granules stain darker than the surrounding cytoplasm The cells, and *especially* the granules, can thus be characterized as argyrophilic, a property which has proved of great value in this study When heart specimens are stained by the Masson-Fontana alkaline silver method for argentaffin substances, the specific cells stain faintly black

After fixation in chrome-containing fixatives the specific cells take on a brownish-yellow colour which must be considered as a positive chromaffin reaction After Orth fixation and subsequent paraffin embedding the chromaffin reaction of the specific cells is weak but if fixation is performed in a mixture of chromate-bichromate and embedding is carried out in polyethylene glycol the positive chromaffin reaction is clearly recognized (Fig 2c)

When stained by the ferric-ferricyanide technique the granular cytoplasm *stains fairly strongly blue-green indicating the presence of reducing* substances The chrome haematoxylin method of BARGMANN (1950) stains the cells in a bluish tone

The osmium-iodide technique of Champy-Coujard stains the specific cells of cyclostome hearts grayish-black With this technique fine cytoplasmic granules are visualized as are a few larger granules which *stain intensely black.*

Fluorescence

Investigations into the fluorescence of the specific cells is rendered difficult by the large amount of pigments in the heart muscle cells These pigments, probably of lipochrome-nature, fluoresce brightly yellow in the technique used The intensity of this fluorescence and the large amounts of pigment present *makes it extremely* difficult to evaluate any fluorescence of the specific cells and thus the fluorescence investigations have been inconclusive

Electron microscopy

Electron micrographs from the hearts of the investigated cyclostomes have yielded detailed *information on the fine* structure of the specific cells encountered (Fig 3) In electron microscopical preparations the cells are conspicuous indeed with their cytoplasm generally almost completely filled with minute, intensely osmiophilic granules of varying size and shape

The granular cells generally show a prominent cell body and peripherally the cell outline varies from a *smooth* elongated shape to rather irregular with

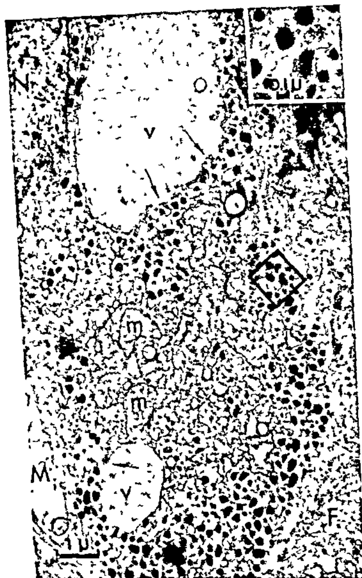


Fig 3 Electron micrograph of a specific cell in *Petromyzon* heart ventricle The cytoplasm is seen to contain both mitochondria (m) and large numbers of dense osmiophilic granules surrounded by membranes Some of the granules show a fine particulate structure (inset) The cell nucleus is seen in the upper left hand corner (N) Two large vacuolar structures (v), surrounded by vesicles and cytoplasmic granules some of which appear to be undergoing structural changes (arrows) are also observed The specific cell borders on a muscle cell (M) and, in the lower right hand corner endomysial collagen fibrils are seen Magnification 12,000 × (inset 30 000 ×)

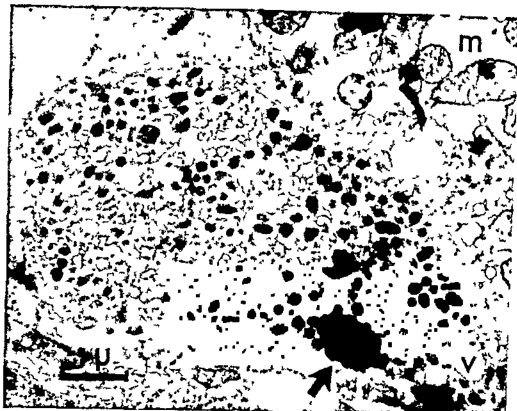


Fig 4 Electron micrograph from *Myxine* ventricle showing a cytoplasmic protrusion of a specific cell in cross section In the cytoplasm both rounded and rod shaped granule profiles are seen Furthermore there appear a large number of seemingly empty cytoplasmic vesicles as well as a vacuolar structure (v) and a large, dense mass (arrow) possibly derived from fused granular material At the upper right is seen part of a heart muscle cell, rich in sarcoplasm and mitochondria (m) Magnification 14,000 ×

*cytoplasmic processes branching from the cell and extending as protrusions be-*tween neighbouring muscle cells Due to the thinness of the sections these fine protrusions can only be followed for a short distance in the electron microscope The protrusions always contain granules

The cells are surrounded by a cell membrane around 50 Å in thickness The prominent nucleus is mostly rounded but can sometimes be extremely irregular in shape The nuclear fine structure after osmium fixation appears granular Rather large granules (200—250 Å) are loosely dispersed throughout the nuclear sap corresponding to the lighter areas of the nucleus In addition considerably smaller granules (around 125 Å) are densely packed together in patchy areas which most often extend to the inner nuclear membrane The nucleus is surrounded by a double-layered nuclear envelope *in which the outer mem-*brane can often be seen to be continuous with membranes of the cell cytoplasm The nuclear membranes are sometimes widely separated resulting in marked perinuclear intramembranous spaces

Fig 5 Electron micrograph of cytoplasm of a specific cell in *Myxine* atrium Osmiophilic, membrane-enclosed granules which almost completely fill the cytoplasm Magnification 27,000 ×



Fig 6 Detail of a specific granular cell in *Petromyzon* atrium This cell is separated from a heart cavity (HC) by a thin cytoplasmic rim of an endothelial cell (E) The specific cell borders directly on a heart muscle cell (M) The typical granules are conspicuous and vacuoles (v) are also observed. *Electron micrograph.* Magnification 22,000 ×



Fig 7 Electron micrograph of a heart muscle cell from *Petromyzon* atrium Myofibrillar elements (f) cytoplasmic vesicles and a large number of mitochondria (m) can be seen in the sarcoplasm Granules of a type similar to those encountered in the specific cells of the heart are also observed (arrows) Magnification 14 000 ×

The cytoplasm of the granular cells shows the common structural components such as mitochondria and endoplasmic reticulum and occasionally a Golgi apparatus can be identified The most conspicuous component however is the cytoplasmic droplets or granules of varying size and shape which in most cases fill the cytoplasm to such an extent as to totally obscure other cytoplasmic structures (Figs 4—6)

The granules are mostly round in shape but elongated rod like granules can also be observed The size of the round granule profiles varies considerably between 100—300 mμ but the majority are of submicroscopic dimensions viz < 200 mμ The granules are mostly surrounded by a limiting surface membrane with a thickness in the order of 50—75 Å This membranous structure is strongly osmiophilic and appears in electron micrographs as a single dense line Inside the granular membrane a peripheral space of lower electron density generally separates the membrane from a central core The light halo varies widely in thickness being barely noticeable in some granules while in others the space may reach a width of 200—300 Å (Figs 4 and 5)



Fig 8



Fig 9

Figs 8—9 Light photomicrograph of *Petromyzon* atrium Fig 8 Normal heart with a number of dark-staining specific cells Fig 9 Specimen from a reserpine-treated animal Only a *few*, poorly stained cells are visible (arrow) Both sections were placed on the same slide and stained simultaneously Comparable heart areas were photographed Modified Palmgren stain. Magnification around 200 ×

The core of the granules is extremely electron dense due to large deposits of reduced osmium In many granules this zone appears homogenous but in others a fine-granular inner structure is seen, these minute granules having diameters of 100—200 Å (Fig 3)

It is not uncommon to find larger, irregular, dense bodies among the granules These may reach sizes of several microns They generally do not show any inner structure and their overall density corresponds very well to that of surrounding granules possibly indicating an origin from fused granular material (Fig 4)

Cytoplasmic vesicles, seemingly empty and surrounded by a membrane, are a common occurrence (Figs 3 and 4) These vesicles are of sizes that correspond to those of granules and probably represent granular envelopes emptied of contents In a few cases large vacuolar structures with diameters up to 6—7 μ have been observed in the cells (Fig 3) Surrounding these vacuoles large numbers of cytoplasmic vesicles can be observed and also granules which appear to be undergoing structural changes The vacuolar contents are finely granular and probably contain substances derived from disrupted and dissolved cytoplasmic granules

Electron micrographs of heart muscle cells from both lamprey and hagfish have also revealed that granules of the same type as described in the specific granular cells can also be found in typical heart muscle cells (Fig 7) When this is the case the granules are situated in the cytoplasm of the muscle cells, either grouped together in small accumulations and surrounded by mitochondria and pigment granules or the granules may be dispersed throughout the sarcoplasm and single granules may even be found between the myofibrillar elements

Effect of reserpine on histology of MYXINE *and* PETROMYZON *hearts*

Subcutaneous injections of reserpine were given over various periods of time Heart specimens from treated animals as well as from untreated controls were prepared for light microscopy Sections from both were mounted on the same slide and stained with the modified Palmgren stain

Examination of the slides showed a marked difference between the two sections (Figs 8 and 9) While in sections from the untreated controls large numbers of specific cells could easily be identified, the same cells were almost impossible to find in those from reserpine treated animals This latter finding proved to be due to a depletion of granules from the specific cells, as evidenced by catecholamine assays of the hearts of similarly treated animals

II *Chromatographic separation of catecholamines in hearts of* MYXINE *and* PETROMYZON

In a previous report figures were given for the content of adrenaline (A) and noradrenaline (NA) in the heart of *Myxine* and *Petromyzon* (ÖSTLUND *et al* 1960) In the following figures are also included assay values obtained after the publication of ÖSTLUND *et al* (1960) Average figures in μg per g for *Myxine* were atrium A, 13, NA, 47, ventricle A, 49, NA, 6 2, portal heart A, 3 4, NA, 53 (atrium and ventricle together A, 36, NA, 16) For *Petromyzon* the corresponding figures were atrium A, 130, NA, 6 3, ventricle A, 28, NA, 0 The whole heart contained on an average A, 51, NA, 1 5 In view of the re-

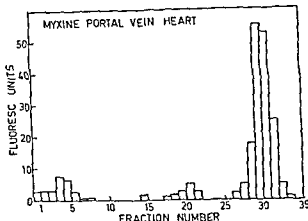


Fig 10 Column chromatogram of alumina eluate of extract of *Myxine* portal vein heart Solvent system n butanol—0 1 N HCl—10 N acetic acid (5 1 2) Aliquots of 2 2 ml fractions condensed with ethylene diamine Fluorescence maximum at the site of noradrenaline Small peaks at the sites of adrenaline and dihydroxy mandelic acid

markably high figures found, which have been confirmed in the present study, extracts were also subjected to starch column chromatography after adsorption on alumina and elution in order to separate the different catechol compounds present

MYXINE *heart*

In chromatograms of extracts of the ventricle of *Myxine* practically all of the fluorescence after condensation with ethylene diamine was found in the region of adrenaline The fractions showing maximal fluorescence were examined spectrophotofluorimetrically and gave the typical maximum at 540—550 mμ for adrenaline, using 410 mμ as exciting wavelength

A chromatogram of the atrium showed a larger peak at the site of noradrenaline and a smaller one at the site of adrenaline, corresponding to the results of fluorimetric estimation of the original extracts according to the routine assay method of EULER and LISHAJKO (1961 a) Spectrophotofluorimetric control of the fractions indicating adrenaline and noradrenaline showed the expected fluorescence maxima

Extracts of the portal vein heart showed a large peak for noradrenaline and a small peak for adrenaline (Fig 10)

PETROMYZON *heart*

An extract from 2 5 g whole heart of *Petromyzon* showed after adsorption on alumina, elution and reextraction in butanol, on chromatography a large fluorescence maximum for adrenaline, and a small fluorescence for noradrenaline, again in agreement with the fluorimetric estimation of the extract as obtained by the routine assay technique mentioned above The small fluorescence

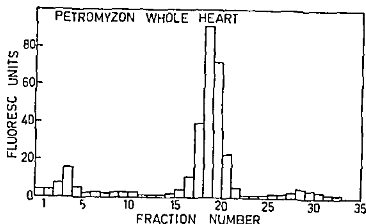


Fig 11 Column chromatography on starch column of extract of whole heart of *Petromyzon* Fluorescence maximum at the site of adrenaline

peak in fraction 4 corresponded spectrophotofluorimetrically to dihydroxyphenylacetic acid (Fig 11)

It is of interest that no evidence was obtained in any of the extracts for the presence of appreciable amounts of dopamine Whether or not other catechol compounds moving more slowly than noradrenaline are present cannot be decided

III. *Assay for histamine and 5-HT in* PETROMYZON *hearts*

When tested on the isolated guinea pig ileum an extract of *Petromyzon* hearts with trichloroacetic acid, from which the acid was removed by ether, showed only a very weak stimulating action corresponding to less than 2 6 μg histamine per g of heart This effect was not abolished by an antihistaminic It can therefore be concluded that the *Petromyzon* heart contains at most very small quantities of histamine This is in agreement with previous results by AUGUSTINSSON *et al* (1956) The possible presence of 5-HT was examined by recording the fluorescence spectra of the acidified eluate from amberlite XE-64 at an excitating wavelength 285 mμ in the Aminco spectrophotofluorimeter The recorded curves show the adrenaline and noradrenaline peak at 335 mμ in the heart extract but no fluorescence at the site of the 5-HT maximum Addition of a small amount of 5-HT to a part of the extract produced the characteristic 5-HT fluorescence peak at 545 mμ (Fig 12) (BOWMAN, CAULFIELD and UDENFRIEND 1955)[1]

The results indicate that the *Petromyzon* heart extract did not contain 5-HT in such amounts that its presence could conceivably interfere with chromaffin cell pictures, since catechol amines were readily demonstrated in large quantities

[1] See also American Instrument Co Bulletin Feb 25, 1957

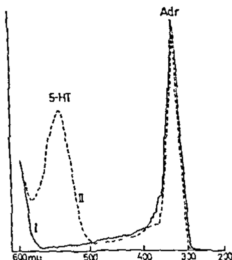


Fig 12 Fluorescence spectra of eluate from amberlite XE-64 of extract of *Petromyzon* heart Exciting wavelength 285 mμ Full line, sample alone Broken line, 5 HT added to sample Fluorescence maxima at 335 mμ (adrenaline and noradrenaline), and at 545 mμ (5-HT)

IV. *Effect of various factors on the catecholamine storage granules from* MYXINE *heart*

Osmotic effects

Granules from *Myxine* hearts were suspended in 0 30 M K-phosphate and resuspended in K-phosphate of varying concentrations As seen in Table I the amounts of catecholamines recovered in the sediment were greatly dependent on the concentration of phosphate

In a concentration of 0 15 M K-phosphate most of the catecholamines were rapidly released and found in the supernatant, while in 0 30—0 38 M solution

TABLE I *Amount of adrenaline and noradrenaline in the resuspended sediment and the supernatant from 1.97 g* MYXINE *hearts at varying concentrations of K-phosphate at pH 7 5 Original suspension contained 0 3 M K-phosphate at pH 7 6 Centrifugation 30 min at 50 000 × g at + 4° C immediately after resuspension*

Resuspended sediment K phosphate conc.	In supernatant μg		In sediment μg	
	Noradr	Adr	Noradr	Adr
0 15 M	5 7	40	1 9	3 8
0 23 M	2.8	18 5	4 4	20 5
0 30 M	0 80	9 0	6.7	29.5
0 38 M	1 1	6 0	5 9	30 3
Original sediment	—	—	8 4	42 5

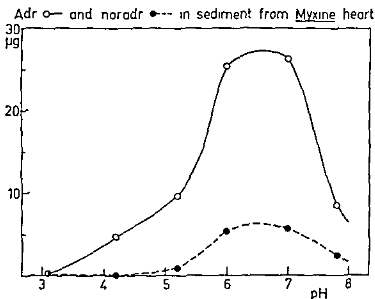


Fig 13 Adrenaline and noradrenaline in isolated granules from 1 8 g *Myxine* heart after sedimentation and resuspension in 0 3 M K phosphate pH adjusted to 3 1—7 8 with phosphoric acid and phosphate buffer

the greater part of adrenaline as well as noradrenaline was recovered in the sediment The low stability in 0 15 M K-phosphate is in marked contrast to that of the granules from the *Petromyzon* heart in which most of the adrenaline is retained in the sediment when the suspension is exposed to 0 1 M K-phosphate and suggests that the *Myxine* granules are adapted to the higher osmotic concentration prevailing in this animal

Effect of acidity, freezing and thawing, detergents

Isolated granules from the adrenal medulla (Hillarp and Nilson 1954) or from bovine splenic nerves (Euler and Lishajko 1961 b) rapidly loose their catecholamine content when exposed to acids at a pH-value of less than 4—5

In the present study sedimented granules from *Myxine* heart were resuspended in 0 30 M K-phosphate, which had been adjusted to pH-values 3 1—7 8 with phosphoric acid, mono- and dihydrogenphosphate The granule suspension was immediately centrifuged for 30 min at 50,000 × *g* at + 4° C and adrenaline and noradrenaline determined in the sediment and in the supernatant

As seen in Fig 13 maximal stability is found at pH 6—7 as for medullary and nerve granules of mammalian origin At pH 4 and even at pH 5 a considerable release occurs and at pH 3 the granules are rapidly and completely emptied of their contents The noradrenaline and the adrenaline curves seem to run parallel, indicating that the two amines are stored in granules with similar properties

Freezing and thawing affects the catecholamine content of adrenal medullary granules quite markedly but has less effect on nerve granules When *Myxine* heart granules were exposed to freezing (— 23° C) and thawing this treatment caused less than 10 per cent release of catecholamines

Detergents are very efficient in depleting the granules from the adrenal medulla and from nerves. After resuspension of the *Myxine* heart granules in 0.30 M K phosphate at pH 7.0, containing 1 mM sodium lauryl sulphonate, only about 20 per cent of the control amount of catecholamines was found in the sediment after centrifugation.

In most experiments the catecholamines were measured also in the supernatant after resedimentation of the granules which had been resuspended in various media. The amounts found in the supernatant corresponded closely to the amounts released from the granules, indicating that in the resuspended sediment there is no efficient inactivating system present.

Spontaneous release

Suspensions of adrenal medullary granules and adrenergic granules show a certain degree of spontaneous release of the catecholamines at room temperature, the rate being considerably higher for the nerve granules, about 80 per cent of the total amount being released in 2 hours. Isolated granules from cyclostome hearts (*Petromyzon*) were found to release only about 30 per cent under the same conditions (Table III).

Proportion of catecholamines occurring in granules

In a previous study it was shown that on an average 28 per cent of the total catecholamine content of bovine splenic nerves could be recovered in the granular sediment, the remainder being found in the supernatant (Euler and Lishajko 1961 b). Of the catecholamines in *Myxine* and *Petromyzon* hearts some 50—70 per cent of the total catecholamine content was found in the sedimented fraction, the proportion being slightly higher for lamprey than for hagfish hearts. Also in this respect the catecholamine stores in cyclostome hearts present greater similarity to the adrenal medullary cells than to adrenergic nerves.

V. *Action of reserpine*

Depletion of catecholamines in Petromyzon *hearts after reserpine*

Preliminary trials showed that lampreys were fairly sensitive to reserpine and generally succumbed within 1—2 hours after doses exceeding 0.3 mg subcutaneously or intramuscularly. Doses of 0.1—0.2 mg were, however, mostly well tolerated and could be repeated daily over a period of many days.

Fig. 14 shows the effect of daily doses of 0.1—0.2 mg reserpine as Serpasil® subcutaneously over various time periods on the catecholamine content of the ventricle and atrium of the lamprey.

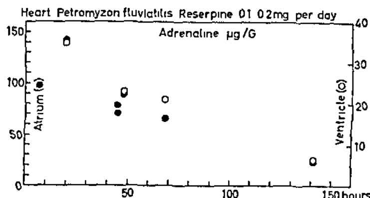


Fig 14 Adrenaline µg/g in ventricle (○ right scale) and atrium (● left scale) of hearts of *Petromyzon* treated various lengths of time with reserpine 0 1—0 2 mg per day

As seen in the figure there is no reduction in the catecholamine content of either the ventricle or the atrium in 20 hours After 48 hours, however, there is a conspicuous fall in both, which is further accentuated after 68 and 141 hours At this time there is only about 15—20 per cent left of the original amount Even after single doses of 0 1 and 0 3 mg allowed to act for 45 hours the fall in catecholamine content of the heart was considerable, the adrenaline content being 20 µg/g and 9 µg/g in the ventricle and 71 µg/g and 78 µg/g in the atrium after 0 1 mg and 0 3 mg reserpine respectively, the normal values being about 30 µg/g for the ventricle and 130 µg/g for the atrium

Effects of reserpine on isolated granules from PETROMYZON *heart*

Granules prepared from 6 whole lamprey hearts were sedimented and resuspended in 7 ml 0 075 M K phosphate and incubated with reserpine phosphate in concentrations of 0 05—0 2 mg/ml for 30 min at + 22—23° C (Table II)

From the table it can be seen that with the high concentrations used, reserpine exerts a certain releasing effect similar to that observed with resuspended granules from mammalian splenic nerves In two other experiments the same kind of action was obtained In a lower concentration range, incubation with reser-

TABLE II *Adrenaline in sediment and supernatant after incubation of resuspended granules from 1 6 g* PETROMYZON *hearts in 0 1 M K phosphate with reserpine (pH 7 0) for 30 min at 22—23° C*

Resuspended sediment	In supernatant µg adr	In sediment µg adr
Controls average	15 3	29 0
Controls range (3)	(15 1 — 15 5)	(28 5 — 29 5)
Reserpine 0 05 mg/ml	20 8	24 2
Reserpine 0 10 mg ml	21 8	23 6
Reserpine 0 20 mg/ml	24 7	21.2

Table III *Adrenaline in the sediment from 1.7 g* Petromyzon *hearts after incubation of the original suspension in 0.13 M K phosphate with reserpine phosphate (pH 6.6) for 2 hours at + 20.5° C*

	Adrenaline µg in sediment
Suspension, incubated 2 hours at + 4° C	56.0
Reserpine 0.03 µg/ml	41.0
Reserpine 0.1 µg/ml	41.8
Reserpine 0.3 µg/ml	40.5
Reserpine 1.0 µg/ml	40.6
Reserpine 3.0 µg/ml	43.5
Reserpine 10 µg/ml	43.5
Control incubated 2 hours at 20.5° C	40.5

pine of the original suspension caused no action in concentrations up to 1 µg/ml but a moderate degree of inhibition of the release when added to 3 and 10 µg/ml (Table III). For comparison it may be recalled that the release from mammalian nerve granules is inhibited to 80—90 per cent by the action of reserpine in concentrations 1—10 µg/ml to a direct suspension (Euler and Lishajko 1961 c).

The inhibitory effect of reserpine in concentrations of 3 and 10 µg/ml is thus very weak as compared with that found for nerve granules. It can also be seen from the table that the spontaneous adrenaline release from the lamprey heart granules during incubation for 2 hours at + 20.5° C is only 28 per cent of the amount present in the control maintained for 2 hours at + 4° C. The heart granules in this respect behave similarly to the medullary granules in bovine and rabbit adrenals, but differently from bovine splenic nerve granules, which give off about 75—80 per cent of their noradrenaline content in 2 hours at 20° C.

Catecholamines in Myxine *heart after reserpine in vivo*

Eight animals received subcutaneous injections of reserpine (Serpasil®) in doses of 0.4—0.85 mg over a period of from 46 to 142 hours. A reduction of the catecholamine content of the heart was found in all the treated animals in comparison with the control values (Fig. 15), although there was no strict correlation between the degree of depletion and the dose or period of treatment. In the four animals which received the largest doses over 126—142 hours the lowest figures were observed however. The effect of reserpine on the hagfish heart is thus similar to that found on the lamprey heart.

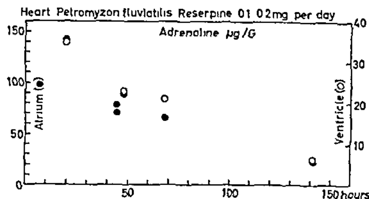


Fig 14 Adrenaline, µg/g in ventricle (○ right scale) and atrium (● left scale) of hearts of *Petromyzon* treated various lengths of time with reserpine 0 1—0 2 mg per day

As seen in the figure there is no reduction in the catecholamine content of either the ventricle or the atrium in 20 hours After 48 hours, however, there is a conspicuous fall in both, which is further accentuated after 68 and 141 hours At this time there is only about 15—20 per cent left of the original amount Even after single doses of 0 1 and 0 3 mg allowed to act for 45 hours the fall in catecholamine content of the heart was considerable, the adrenaline content being 20 µg/g and 9 µg/g in the ventricle and 71 µg/g and 78 µg/g in the atrium after 0 1 mg and 0 3 mg reserpine respectively, the normal values being about 30 µg/g for the ventricle and 130 µg/g for the atrium

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From the table it can be seen that with the high concentrations used, reserpine exerts a certain releasing effect similar to that observed with resuspended granules from mammalian splenic nerves In two other experiments the same kind of action was obtained In a lower concentration range, incubation with reser-

TABLE II *Adrenaline in sediment and supernatant after incubation of resuspended granules from 1.6 g* PETROMYZON *hearts in 0 1 M K phosphate with reserpine (pH 7 0) for 30 min at 22—23° C*

Resuspended sediment	In supernatant µg adr	In sediment µg adr
Controls average	15 3	29 0
Controls range (3)	(15 1 – 15 5)	(28 5 – 29 5)
Reserpine 0 05 mg/ml	20 8	24.2
Reserpine 0 10 mg/ml	21 8	23 6
Reserpine 0 20 mg/ml	24 7	21.2

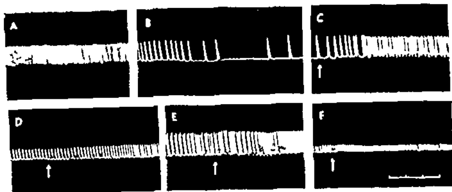


Fig 16 A—D Isolated *Myxine* heart perfused with 2/3 sea water Reserpine 2 5 μg ml in perfusion fluid

A Beginning of perfusion Rate 42/min

B 10 min after start of perfusion

C. Infusion of noradrenaline 1 μg/ml (at arrow), 13 min after start of perfusion Increase in heart rate

D 30 min later Noradrenaline 1 μg ml added to perfusion fluid at arrow Increase of heart rate from 15 to 22 beats per min

E—F Another heart same conditions

E At arrow noradrenaline 2 μg ml added to perfusion fluid Regularization of heart beats

F Increase of heart rate after addition of 10 μg adrenaline to perfusion fluid Time scale, 20 sec marks

VI *Action of catecholamines on the perfused* MYXINE *heart*

It was shown by ÖSTLUND (1954) that the isolated heart of *Myxine* is insensitive to adrenaline and noradrenaline in concentrations of 5 $\times$ 10^{-7} g/ml which stimulate the heart of most other species FÄNGE and ÖSTLUND (1954) were able to show that, occasionally, noradrenaline produced a periodic block or in some cases a very slight increase in frequency After dihydroergotamine (1 100,000) noradrenaline (10^{-6} g/ml) caused a positive chronotropic and inotropic effect

this kind were made with freshly excised *Myxine* hearts perfused with a mixture of 2 parts of sea water and 1 part of distilled water according to FÄNGE and ÖSTLUND (1954) Reserpine was added to the perfusion fluid to a concentration of 2 5 μg/ml, a concentration which was found to exert a definite effect.

Varying times after the start of the perfusion with reserpine most hearts showed a decrease in frequency, on some occasions developing into a complete standstill (Fig 16 A B) Fig 16 C shows that when noradrenaline 1 μg/ml was added to the perfusion fluid the heart resumed its beating This could be re-

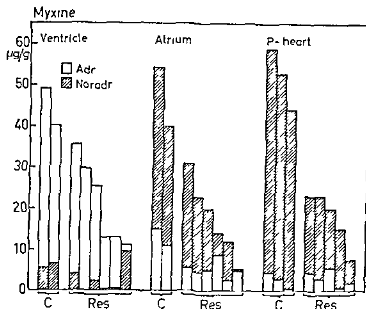


Fig 15 Adrenaline and noradrenaline, μg/g in ventricle atrium and portal venous heart of *Myxine* C controls *RES*, animals treated with reserpine, 0 1—0 2 mg per day for 1—7 days Each column represents one assay

Effect of reserpine on isolated granules from the MYXINE *heart*

The effect of reserpine on the release of catecholamines from isolated resuspended granules from the whole *Myxine* heart including the portal heart was also studied The result (Table IV) shows a somewhat stronger releasing effect of reserpine than that found with isolated granules from lamprey heart, about 70 per cent being released by reserpine 0 15 mg/ml in 30 min as against a maximal release of 28 per cent by 0 2 mg/ml in lamprey heart granules

The releasing effect of high concentrations of reserpine can be observed for noradrenaline as well as for adrenaline The released amounts were almost quantitatively recovered in the supernatant

TABLE IV *Noradrenaline and adrenaline in the granules from 500 mg* MYXINE *heart (including the portal heart) after incubation of resuspended granules for 30 min at pH 6 1 and + 23° C*

	In sediment μg	
	Noradr	Adr
Control	1 9	4 6
Reserpine 0 05 mg ml	1 9	4 2
Reserpine 0 1 mg/ml	0 97	2 1
Reserpine 0 15 mg/ml	0 43	1 7
Control	2 6	5 3

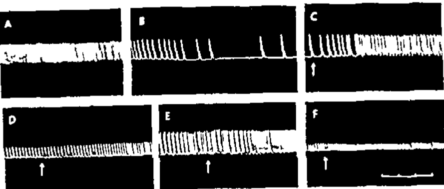


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A Beginning of perfusion Rate 42/min

B 10 min after start of perfus on

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It appeared of interest to examine whether the catecholamines might become active after pretreatment of the isolated heart with reserpine Experiments of this kind were made with freshly excised *Myxine* hearts perfused with a mixture of 2 parts of sea water and 1 part of distilled water according to Fänge and Östlund (1954) Reserpine was added to the perfusion fluid to a concentration of 2 5 μg/ml, a concentration which was found to exert a definite effect

Varying times after the start of the perfusion with reserpine most hearts showed a decrease in frequency, on some occasions developing into a complete standstill (Fig 16 A, B) Fig 16 C shows that when noradrenaline 1 μg/ml was added to the perfusion fluid the heart resumed its beating This could be re-

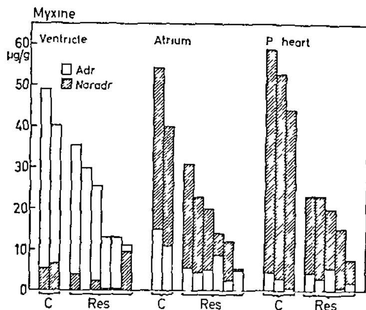


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	Noradr	Adr
Control	1 9	4 6
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Reserpine 0 1 mg/ml	0 97	2 1
Reserpine 0 15 mg/ml	0 43	1 7
Control	2 6	5 3

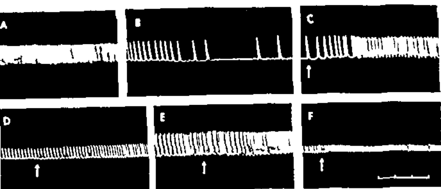


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[illegible] this kind were made with freshly excised *Myxine* hearts perfused with a mixture of 2 parts of sea water and 1 part of distilled water according to FÄNGE and ÖSTLUND (1954) Reserpine was added to the perfusion fluid to a concentration of 2 5 μg/ml, a concentration which was found to exert a definite effect

Varying times after the start of the perfusion with reserpine most hearts showed a decrease in frequency, on some occasions developing into a complete standstill (Fig 16 A, B) Fig 16 C shows that when noradrenaline 1 μg/ml was added to the perfusion fluid the heart resumed its beating This could be re-

peated after washing away the noradrenaline and letting the reserpine exert its action anew On some occasions noradrenaline caused a definite increase in frequency and amplitude in the reserpine treated preparation (Fig 16 D) Adrenaline also increased the frequency of the heart beat after reserpine (Fig 16 F)

It was also noted that noradrenaline was able to regularize the heart beat in such cases where disturbances in conduction resulting in periodic block had appeared during reserpine action (Fig 16 E) On the other occasions the addition of noradrenaline to the perfusion fluid could produce a temporary block by itself as previously noted by FANGE and ÖSTLUND (1954)

DISCUSSION

The occurrence of large amounts of catecholamines in cyclostome hearts, first demonstrated by ÖSTLUND (1954), raises the interesting question in which cell element the amines are stored The high quantity seems to preclude their presence in adrenergic nerves and makes it appear more probable that they are stored in some kind of special cell, perhaps similar to the chromaffin cells of the adrenal medulla By the use of suitable histological and electron microscopical techniques this assumption has proved to be correct Methods employed in this investigation have revealed the presence in the hearts of the two studied cyclostomes, lamprey and hagfish, of a great number of specific granular cells which show a striking resemblance to certain other catecholamine containing cells which have been reported on in the literature

While an early electron microscopical investigation (BLOOM 1960, unpublished) showed the presence of a specific cell type in these hearts it was only after a careful histological investigation that the full extent of this specific cellular system was revealed in the light microscope

In the extensive investigation by AUGUSTINSSON *et al* (1956) these authors could confirm the findings of among others RANSOM and THOMPSON (1886), and JULIN (1887) that the lamprey heart is innervated by fibres originating in the vagus nerve Furthermore they found that the observations earlier made by GREENE (1902) and CARLSON (1904) on the absence of cardioregulatory nerves in the cyclostome *Bdellostoma* also are valid for *Myxine* This is in agreement with findings in the present investigation in which nerve fibres have occasionally been observed in *Petromyzon* but as yet none have been seen in *Myxine*

Chromaffin cells have earlier been reported in *Petromyzon* GIACOMINI (1902) and GASKELL (1912) described the occurrence of chromaffin cells along the cardinal veins in this species and GASKELL also observed cells exhibiting a chromaffin reaction in the wall of the sinus venosus

AUGUSTINSSON *et al* (1956) found that all parts of the lamprey heart contain numerous cells which were considered by these authors to be ganglion cells and were referred to as such They also reported two types of these cells, a small, common type and one which was larger and rather rare They described this system of ganglion cells as forming a continuum throughout the whole heart The so called ganglion cells were granular in nature and were stainable by silver impregnation They showed a chromaffin reaction and the granules stained dark blue with the chromium haematoxylin phloxin method of GOMORI (1941) It is of interest to note that these authors did not observe any similar cells in the heart of *Myxine*

After publication of the report by ÖSTLUND *et al* (1960) there appeared a paper by JOHNELS and PALMGREN (1960) in which these authors upon reinvestigation of *Myxine* heart also observed cells which were stainable by silver impregnation and which these authors suggested may be chromaffin cells

There is little doubt at present that the cells in *Petromyzon* heart termed ganglion cells by AUGUSTINSSON *et al* (1956) and the cells in *Myxine* heart supposed to be chromaffin by JOHNELS and PALMGREN (1960) are in fact the specific granular catecholamine containing cells of both species first reported by ÖSTLUND *et al* (1960) and further investigated in the present study It cannot, however, be excluded that some of the larger cells observed in *Petromyzon* may be true ganglion cells

Silver staining methods have been widely used in histology and there are at present available a variety of such methods with a great number of modifications While some authors consider these staining methods to be specific for certain tissue structures PALMGREN (1960) emphasizes that almost every tissue element is silver stainable

The silver staining method of PALMGREN (1960) originally designed for the staining of nerve fibres can be modified to demonstrate the specific cells in a beautiful way (JOHNELS and PALMGREN 1960) This method is a classical silver impregnation method where after combining with tissue substances reduced silver ions are replaced by gold particles As an extraneous reducer is introduced into the system it is of importance to emphasize that the method is a pure impregnation method which will show up argyrophilic substances but not necessarily argentaffin ones While this method has proved to be valuable for demonstrating the specific cells it does not permit any conclusions to be drawn regarding the nature of the substances stored or produced in the cells

The actual value of the chromaffin reaction in histochemistry has been much discussed (COUPLAND 1954, HALE 1958 PEARSE 1960) The general opinion is that not only do the sites of brownish staining represent deposits of reduced chromium oxide but also the sites of formation of brown pigments through the oxidation of a variety of easily oxidized substances among these the catechol amines While the chromaffin reaction cannot be considered as a specific stain in any way it still retains an undisputable value for localizing *e g* phenolic amines

The specific cells here studied showed a positive chromaffin reaction After fixation in Orth s solution and embedding in paraffin the reaction was weak This is possibly due to the deleterious effect of hot paraffin on the chromaffin reaction (FALCK and HILLARP 1959) Of the techniques used for chromaffinity the chromate bichromate fixation of HILLARP and HOKFELT (1955) proved to be superior especially when followed by embedding in polyethylene glycol as recommended by FALCK and HILLARP (1959)

The Schmorl ferric ferricyanide reaction has been widely used especially in the study of pigments In certain stages of the oxidation of the latter groups

are formed which can reduce a fresh solution of ferricyanide, which then combines with ferric iron to give a precipitate of Prussian blue The method has been extensively investigated by ADAMS (1956) It must be emphasized that this rather simple test will only show the presence of reducing groups but such groups are rather widely distributed in tissues and can also easily be obtained by certain treatments Thus a positive reaction may be obtained by *e g* lipofuscin melanin, argentaffin granules, chromaffin granules and protein structures containing SH groups After fixation in Helly's fluid the specific cells show a general diffuse bluish colour while fixation in formalin brings out granular structures in the cytoplasm to which the blue colour is concentrated

The osmium iodide technique of CHAMPY-COUJARD (1941) was claimed by these authors to be specific for diphenols (sympathin") This opinion has been severely criticized by later investigators (STÖHR 1952, JABONERO 1955 a o) The present authors have also found the method to be unspecific but it will stain the specific cells

That catecholamines can be stored within cells in droplets or granular structures is now a well accepted fact This has also proved to be valid for other monoamines such as 5-hydroxytryptamine, histamine and dopamine As such storage structures often are of small dimensions, light microscopy has not been able to provide much information about them However, the ultrastructure of catecholamine containing adrenal medullary cells from a variety of mammals has now been investigated by a number of authors (LEVER 1955, SJÖSTRAND and WETZSTEIN 1956, DE ROBERTIS and FERREIRA 1957, WETZSTEIN 1957, KLEINSCHMIDT and SCHUMANN 1961, a o)

At least two types of medullary granular cells have been described LEVER (1955) observed both a dark cell with an abundance of osmiophilic granules and a light cell containing fewer secretory granules WETZSTEIN (1957) also recognizes two celltypes which he calls Hauptzellen" and Nebenzellen"

In our preliminary observations a somewhat varying appearance of the specific cells was also noticed Our extended investigation has as yet not proved that a differentiation of cell types is justified In certain cases the variation has been found to depend on the preparatory techniques used while in others it cannot be excluded that the varying appearance simply reflects different stages of functional activity of the cells Our findings are in good agreement with those of LEVER, LEWIS and BOYD (1959) who among the chromaffin cells of the carotid body found cells with a variety of appearances extending from virtually agranular cells with a low density cytoplasm to those with a high general density and granule content

The size of adrenal medullary granules has been stated as 175 mμ in mouse (SJOSTRAND and WETZSTEIN 1956), 160 mμ in rabbit (DE ROBERTIS and FERREIRA 1957) and 150—170 mμ in rat as measured in electron micrographs published by LEVER (1955) In a large number of measurements of the granules of the specific cells of cyclostome hearts a mean granule diameter of around

After publication of the report by ÖSTLUND *et al* (1960) there appeared a paper by JOHNELS and PALMGREN (1960) in which these authors upon reinvestigation of *Myxine* heart also observed cells which were stainable by silver impregnation and which these authors suggested may be "chromaffin" cells

There is little doubt at present that the cells in *Petromyzon* heart termed "ganglion cells" by AUGUSTINSSON *et al* (1956) and the cells in *Myxine* heart supposed to be "chromaffin" by JOHNELS and PALMGREN (1960) are in fact the specific granular catecholamine-containing cells of both species first reported by ÖSTLUND *et al* (1960) and further investigated in the present study It cannot, however, be excluded that some of the larger cells observed in *Petromyzon* may be true ganglion cells

Silver staining methods have been widely used in histology and there are at present available a variety of such methods with a great number of modifications While some authors consider these staining methods to be "specific" for certain tissue structures, PALMGREN (1960) emphasizes that almost every tissue element is silver stainable

The silver staining method of PALMGREN (1960) originally designed for the staining of nerve fibres can be modified to demonstrate the specific cells in a beautiful way (JOHNELS and PALMGREN 1960) This method is a classical silver impregnation method where after combining with tissue substances, reduced silver ions are replaced by gold particles As an extraneous reducer is introduced into the system, it is of importance to emphasize that the method is a pure impregnation method which will show up argyrophilic substances but not necessarily argentaffin ones While this method has proved to be valuable for demonstrating the specific cells, it does not permit any conclusions to be drawn regarding the nature of the substances stored or produced in the cells

The actual value of the chromaffin reaction in histochemistry has been much discussed (COUPLAND 1954, HALE 1958, PEARSE 1960) The general opinion is that not only do the sites of brownish staining represent deposits of reduced chromium oxide but also the sites of formation of brown pigments through the oxidation of a variety of easily oxidized substances among these the catecholamines While the chromaffin reaction cannot be considered as a specific stain in any way it still retains an undisputable value for localizing *e g* phenolic amines

The specific cells here studied showed a positive chromaffin reaction After fixation in Orth's solution and embedding in paraffin the reaction was weak This is possibly due to the deleterious effect of hot paraffin on the chromaffin reaction (FALCK and HILLARP 1959) Of the techniques used for chromaffinity the chromate bichromate fixation of HILLARP and HOKFELT (1955) proved to be superior, especially when followed by embedding in polyethylene glycol as recommended by FALCK and HILLARP (1959)

The Schmorl ferric ferricyanide reaction has been widely used, especially in the study of pigments In certain stages of the oxidation of the latter, groups

are formed which can reduce a fresh solution of ferricyanide, which then combines with ferric iron to give a precipitate of Prussian blue The method has been extensively investigated by ADAMS (1956) It must be emphasized that this rather simple test will only show the presence of reducing groups but such groups are rather widely distributed in tissues and can also easily be obtained by certain treatments Thus a positive reaction may be obtained by *e g* lipofuscin melanin, argentaffin granules, chromaffin granules and protein structures containing SH groups After fixation in Helly's fluid the specific cells show a general diffuse bluish colour while fixation in formalin brings out granular structures in the cytoplasm to which the blue colour is concentrated

The osmium iodide technique of CHAMPY-COUJARD (1941) was claimed by these authors to be specific for diphenols ("sympathin") This opinion has been severely criticized by later investigators (STOHR 1952, JABONERO 1955 a o) The present authors have also found the method to be unspecific but it will stain the specific cells

That catecholamines can be stored within cells in droplets or granular structures is now a well accepted fact This has also proved to be valid for other monoamines such as 5 hydroxytryptamine, histamine and dopamine As such storage structures often are of small dimensions, light microscopy has not been able to provide much information about them However, the ultrastructure of catecholamine containing adrenal medullary cells from a variety of mammals has now been investigated by a number of authors (LEVER 1955, SJOSTRAND and WETZSTEIN 1956, DE ROBERTIS and FERREIRA 1957, WETZSTEIN 1957, KLEINSCHMIDT and SCHUMANN 1961, a o)

At least two types of medullary granular cells have been described LEVER (1955) observed both a dark cell with an abundance of osmiophilic granules and a light cell containing fewer secretory granules WETZSTEIN (1957) also recognizes two celltypes which he calls "Hauptzellen" and "Nebenzellen"

In our preliminary observations a somewhat varying appearance of the specific cells was also noticed Our extended investigation has as yet not proved that a differentiation of cell types is justified In certain cases the variation has been found to depend on the preparatory techniques used while in others it cannot be excluded that the varying appearance simply reflects different stages of functional activity of the cells Our findings are in good agreement with those of LEVER, LEWIS and BOYD (1959) who among the chromaffin cells of the carotid body found cells with a variety of appearances extending from virtually agranular cells with a low density cytoplasm to those with a high general density and granule content

The size of adrenal medullary granules has been stated as 175 mμ in mouse (SJOSTRAND and WETZSTEIN 1956), 160 mμ in rabbit (DE ROBERTIS and FERREIRA 1957) and 150—170 mμ in rat as measured in electron micrographs published by LEVER (1955) In a large number of measurements of the granules of the specific cells of cyclostome hearts a mean granule diameter of around

150 mμ was obtained though large variations are common (100—300 mμ) and granules as small as 40 mμ have occasionally been observed

A membrane surrounding adrenal medullary granules has been reported by all electron microscopists who have studied them While LEVER (1955) mentions a delicate membrane surrounding the granules, SJOSTRAND and WETZSTEIN (1956) measured and reported a 100 Å thick membrane DE ROBERTIS and FERREIRA (1957) recognize a single surface membrane of the order of 30 Å In our specific cells a single limiting membrane around 50—75 Å is found surrounding the individual granules

A light space, 60—200 Å in width, located immediately below the granule membrane has been found to be of common occurrence in granules of the adrenal medullary cells (SJOSTRAND and WETZSTEIN 1956, DE ROBERTIS and FERREIRA 1957, WETZSTEIN 1957) This appears also to be the case in the granules of the specific cells though the variation in width appears to be somewhat greater Thus granules may be found in which practically no space at all is recognized as well as others which exhibit a light peripheral zone with a thickness of 200—300 Å

In the central dense core of the granules of the specific cells minute particles with dimensions of 100—200 Å are sometimes seen A similar structure is observed in adrenal medullary granules where these fine particles have a size of 180—200 Å in rats (as measured in electron micrographs of LEVER (1955)), and 175 Å in mouse (SJOSTRAND and WETZSTEIN 1956) DE ROBERTIS and FERREIRA (1957) describe a fine granular inner structure of the large granules but do not present any measurements

There is thus a definite resemblance between the specific cells of cyclostome hearts and the catecholamine-containing cells of the adrenal medulla of higher vertebrates and especially the ultrastructure shows a remarkable similarity

It is also of great interest to note the agreement between the fine structure of the specific cells and that of the chromaffin cells of the carotid body in cat and rat (LEVER *et al* 1959, ROSS 1959) There are, however, certain discrepancies between the ultrastructure of the here described heart cells and certain other reported chromaffin cells, e g those in human skin described by RHODIN, ADAMS-RAY and NORDENSTAM (1959), and by PHILLIPS, BURCH and HIBBS (1960)

The finding within heart muscle cells of submicroscopical granules seemingly identical with those of the specific cells is highly interesting but difficult to evaluate While it would seem logical to assume that these granules may originate in the specific cells and then pass over to the muscle cells it is at present neither possible to draw conclusions as to the actual mechanism of such a transport nor to postulate a definite function for these granules The observations call for further investigation and special attention is being devoted to this problem

As might be surmised, reserpine treatment of the animal greatly reduces the catecholamine stores of the hearts At the same time the staining of the gran-

ulated cells decreases strongly, indicating that these cells are in fact storage cells for catecholamines A strong support for this conclusion is further that granules rich in catecholamines can be isolated from the hearts These granules show many properties in common with the adrenal medullary granules and the adrenergic nerve granules The slow spontaneous release rate of the catecholamine as well as the relatively high proportion of total catecholamines which can be recovered in the granules from the cyclostome hearts agrees more with the adrenal medullary granules than with nerve axon granules Similarly the relatively weak action of reserpine on the isolated granules is in better accord with the results obtained on medullary granules than on nerve granules (EULER and LISHAJKO 1961 c)

Of certain interest is the different osmotic susceptibility of the granules from *Petromyzon* and *Myxine*, the latter animal showing isotonicity at a higher osmolar

art of cyclo-

Addition of

reserpine to the perfusion fluid of the isolated heart caused a reduction in heart rate even to a stand still of the heart Under these conditions, however, a definite positive chronotropic and inotropic effect could be observed both for adrenaline and noradrenaline It is tempting to relate the large amounts of catecholamines present in the heart to the weak action of catecholamines added to the normal cyclostome heart The functional importance of the large amounts of catecholamines in the cyclostome heart is still obscure but it appears possible that they are related to some metabolic action

The finding of a system of specific catecholamine containing cells described in this paper has helped to explain the high catecholamine values found in cyclostome hearts It is only logical to assume that cells of a similar type may also occur in other invertebrate or vertebrate organs containing catecholamines

Whether the chromaffin cell system here described subserves the functional needs of the heart alone or whether the hormonal products released exert systemic actions cannot be stated at present In the latter case the cromaffin cell accumulation in the cyclostome heart may correspond functionally to the chromaffin cell groups of glandular character in other species

SUMMARY

Heart tissue of the cyclostomes *Petromyzon fluviatilis* (lamprey) and *Myxine glutinosa* (hagfish) contains large amounts of adrenaline and noradrenaline which are characteristically distributed in the different parts of the heart A combined light and electron microscopical study of the hearts of the two cyclostomes has revealed the presence of a large number of specific granular cells similar to those of the adrenal medulla in higher vertebrates The cell granules which are regarded as storage sites for catecholamines are strongly osmiophilic and have a diameter of about 0 15 μ The specific cells and the granules are described in detail as well as their appearance after various staining procedures Isolated granules show properties similar to those of adrenal medullary cells but differ in some respects from those of adrenergic nerves

The distribution of noradrenaline and adrenaline in the different parts of the heart of *Myxine* and *Petromyzon* has been verified by chromatographic separation Only small amounts of other catechol compounds have been found in extracts of these hearts

Tests for 5-HT and histamine in extracts of the cyclostome hearts showed that these amines were not present in significant amounts

Reserpine treatment of the animals causes considerable depletion of the catecholamine stores of the heart On isolated granules reserpine causes increased release in higher and inhibition of release in lower concentrations The hearts from reserpine treated animals show only a very weak staining of the granular cells

Cyclostome hearts are almost insensitive to catecholamine but after pretreatment with reserpine a stimulating action of these amines could be demonstrated

ADDENDUM

After this paper was prepared a paper by D Jensen, division of Marine Biology University of California U S A, was brought to our attention dealing with the cardioregulation in the hearthit of the California hagfish *(Eptate rus stoutii)* (Comp Biochem Physiol 1961 2 181—201) This author has also found granular cells in the heart of this species and his histological *findings strongly corroborate* those of the present authors The nature of the cardio accelera tory substance found in the heart could not be decided so far, however

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Contents

Introduction

In the recent era of research on spinal cord organization the main emphasis has been on the connexions of the large muscle afferents whereas the flexion reflex actions have not received so much attention. It should be remembered, however, that SHERRINGTON's investigations on the flexion reflex remain the basis for important principles in spinal cord organization. The concept of reciprocal innervation arose from the findings on the flexion reflex actions and was formulated by SHERRINGTON (1906): the reflex inhibition (relaxation) and the reflex excitation (contraction) are part and parcel of one and the same reflex reaction and that although opposite in direction they are coordinate reciprocal factors in one united response. In further investigations SHERRINGTON (1910) classified muscles as flexors or extensors partly depending on whether they contracted or relaxed during the flexor reflex evoked by a standard test stimulus.

In electrophysiological experiments it has been shown that excitatory action is evoked in flexor and reciprocally inhibitory action in extensor alpha motoneurones by impulses in group II and III muscle afferents (LLOYD 1943, 1946; BROCK, ECCLES and RALL 1951; LAPORTE and LLOYD 1952; LAPORTE and BESSOU 1959; ECCLES and LUNDBERG 1959 a, b; PAINTAL 1961), in cutaneous afferents (LLOYD 1943; HAGBARTH 1952; LAPORTE and BESSOU 1958; ECCLES and LUNDBERG 1959 a, b) and in high threshold joint afferents (ECCLES and LUNDBERG 1959 a, b). These afferents have been denoted the *flexion reflex afferents* (*FRA*) (ECCLES and LUNDBERG 1959 a; HOLMQVIST, LUNDBERG and OSCARSSON 1960).

In order to appreciate the functional significance of the flexion reflex actions it has proved necessary to take into account the supraspinal control of their reflex arcs. In the decerebrate cat the synaptic actions of the FRA are small or absent (JOB 1953; ECCLES and LUNDBERG 1959 b; HOLMQVIST and LUNDBERG 1959 a, b; KUNO and PERL 1960) because of a tonic inhibitory control from supraspinal centres of the interneurones mediating the actions from the flexion reflex afferents. A similar control has been found of the Ib pathways but not of the Ia inhibitory pathway (ECCLES and LUNDBERG 1959 b). Some aspects of the organization of this

When relying exclusively on the technique of conditioning monosynaptic reflexes for disclosure of excitability changes in motoneurones great caution must be taken when investigating group I synaptic actions from flexor muscles because they provide presynaptic inhibition of the monosynaptic Ia pathway (FRANK and FUORTES 1957, ECCLES *et al* 1960) On the other hand ECCLES *et al* (1960) found no or little evidence for presynaptic inhibition of the monosynaptic Ia pathway from high threshold muscle afferents and the technique of conditioning monosynaptic reflexes should be satisfactory for revealing excitability changes evoked in motoneurones from these afferents

In connexion with the existence of presynaptic inhibition it is of interest that KUNO and PERL (1960) have claimed that in the decerebrate state volleys in group II and III afferents from BSt inhibit test reflexes from gastrocnemius soleus In our experience group II and III effects from the BSt nerve are equally well suppressed in the decerebrate state as the corresponding effects from other nerves In similar experiments on decerebrate cats R M ECCLES and LUNDBERG (unpublished) sometimes found inhibition of gastrocnemius soleus and flexor digitorum longus test reflexes from posterior biceps semitendinosus but evoked exclusively by group I volleys It was confirmed by intracellular recording that this inhibition was presynaptic, *i e* group I volleys from BSt did not evoke any synaptic action in gastrocnemius soleus motoneurones whereas the homonymous monosynaptic EPSP was decreased The inhibitory effect from BSt described by KUNO and PERL (1960) has the latency and initial time course of presynaptic inhibition We do assume that it is a group I effect and that they have failed to differentiate between effects evoked by low and high threshold muscle afferents

The mechanism by which supraspinal centres exert control of spinal reflex arcs also deserves comment Since in the decerebrate state volleys *in certain* afferents failed to evoke synaptic actions in motoneurones it was postulated that interneurones of their pathways were inhibited from the brain stem (ECCLES and LUNDBERG 1959 b) Support for this postulate is provided by the observation that activation of the inhibitory supraspinal control system evokes large inhibitory potentials in interneurones (in and around the intermediate nucleus) which can be excited by volleys in somatic afferents (LUNDBERG and VOORHOEVE 1961 b) On stimulation of the sensory motor cortex on the other hand excitatory post synaptic potentials (EPSPs) are evoked in these interneurones and correspondingly many spinal reflex arcs are facilitated by pyramidal activation (LUNDBERG and VOORHOEVE 1961 a)

inhibitory controlling system in the cat has been revealed (Holmqvist and Lundberg 1959 a). The descending pathway is located in the dorsal part of the lateral funicles and from each side a bilateral effect is exerted. The centres maintaining the inhibition of the interneurones of these reflex arcs can operate independently of those responsible for decerebrate rigidity, as is also shown by the fact that decerebrate rigidity depends on ventral pathways (Fulton, Liddell and Rioch 1930, Downman and *Hussain 1958*). The control of spinal interneurones mediating the actions of the flexion reflex is exerted from medially located brain stem centres: it remains after a bilateral removal of the vestibular nuclei but disappears after a medial lesion at obex (Downman and Hussain 1958, Holmqvist and Lundberg 1959 a).

For the further analysis we wanted to learn at which level the responsible centres were located and transverse lesions were made at progressively more caudal levels in the medial brain stem. The effects of these *lesions will be described in this paper. It has turned out that the control* mechanism is complex and indicating new interesting principles for the organization of spinal reflexes. There is evidence of independent control of the reciprocal actions to extensor and flexor motoneurones and equally important is the finding that after a low pontine lesion impulses in the FRA evoke inhibition in flexor motoneurones (*cf.* Eccles and Lundberg 1959 a). A preliminary report of some of the findings has been published (Holmqvist and Lundberg 1959 b).

Remarks on the synaptic actions exerted by the FRA and the supraspinal control of reflex arcs

When using the technique of conditioning monosynaptic reflexes it is important to realize that there are different mechanisms whereby a conditioning volley may change the size of a monosynaptic test reflex. *A conditioning volley may* evoke excitatory or inhibitory synaptic actions in motoneurones (Brock, Coombs and Eccles 1952, Coombs, Eccles and Fatt 1955 a, b), thereby changing the *excitability of the motoneurones and the size* of the monosynaptic reflexes. On the other hand a conditioning volley may depress a monosynaptic test reflex on the presynaptic side (Frank and Fuortes 1957, Frank 1959, Eccles, Eccles and Magni 1960, Eccles 1961). In this case the reflex is depressed even when the conditioning volley does not exert any recordable synaptic action *in the* motoneurones, presumably because the conditioning volley depolarizes the presynaptic fibres in the testing reflex arc (Eccles *et al.*, 1960).

The investigations by Eccles and *Lundberg* (1959 b) and Holmqvist and Lundberg (1959 b) on the supraspinal control of reflex arcs have been concerned with the former actions and not with presynaptic inhibition as has been proved *in parallel investigations* with intracellular recording of post synaptic potentials

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Methods

The experiments were when not otherwise stated made on unanaesthet decerebrate decerebellectomized cats Ether was used during the operation was administered in suitable mixture with air and oxygen to give a cons depth of anaesthesia Recording was started 2 hours after withdrawal of et During the experiment the animals were immobilized with Flaxedil and artifici respirated The blood pressure was continuously measured in most of the exp ments and intravenous infusion of Dextran *given if there were signs of circul* failure

In most of the experiments the synaptic actions were investigated by con tioning monosynaptic test reflexes from posterior biceps semitendinosus gastr nemius soleus and tibialis anterior + extensor digitorum longus (deep perone To evoke monosynaptic reflexes double stimuli were often given the strength the first stimulus was subliminal for discharge of the motoneurones but facilita the second volley (ECCLES and LUNDBERG 1959 b) The advantage of this te nique is that relatively large monosynaptic reflexes can be obtained and the s of the reflex can easily be adjusted After pontine lesions the excitability of alpha motoneurones increases considerably and to equal the size of mo synaptic reflex evoked by double volleys in the decerebrate cerebellectomi state it was after a low pontine lesion often sufficient to use single submaxi la stimulation In most of the experiments the monosynaptic reflexes w recorded from the ventral roots but sometimes recording was made from peripheral muscle nerve If so the ventral roots were intact and the reflex evok by a single volley in the appropriate dorsal root

For conditioning single stimuli were given to various muscle and cutaneo nerves and to the posterior knee joint nerve Condenser discharges (half time decay 45 μ sec) *were used for stimulation Even at maximal strength these puls* did not activate C fibres 4—6 different conditioning strengths were general used for each muscle nerve and the strength always measured relative to thresho strength for the nerve (*cf* ECCLES and LUNDBERG 1959 b) At each streng usually 10—15 different intervals between conditioning and testing volley we selected and 10—20 superimposed records were taken (*cf* Fig 1)

In some experiments a simplified technique was applied to measure the effe of a conditioning volley by superimposing about 200 monosynaptic test refle *in one photographic exposure (cf* Fig 7) In order to obtain a scatter of the trace on the records the test reflex was automatically delayed in successive sweeps s as to traverse the screen and for each record this procedure was repeated 4— times The CRO brightness of the baseline was kept very weak and brightenin pulses were applied to the grid of the CRO during the discharges The super

mposed unconditioned test reflexes give a band of a certain width and the effect of a conditioning volley given at a fixed interval after the sweep start is displayed as a widening (facilitation) or narrowing (inhibition) of the band.

Intracellular recording from motoneurones was made as described by BROCK *et al* (195?) with microelectrodes filled with 3 M KCl or 0 6 M K_2SO_4

Brain stem lesions were made with a 3 mm wide spatula perpendicularly from the floor of the 4th ventricle and always through the entire medial brain stem. After the experiment the brain stem was fixed in 10 per cent formalin. Lesions in the spinal cord were controlled histologically as described by LAPORTE, LUNDBERG and OSCARSSON (1956 a)

Methods

The experiments were, when not otherwise stated, made on unanaesthetized decerebrate decerebellectomized cats Ether was used during the operation and was administered in suitable mixture with air and oxygen to give a constant depth of anaesthesia Recording was started 2 hours after withdrawal of ether During the experiment the animals were immobilized with Flaxedil and artificially respirated The blood pressure was continuously measured in most of the experiments and intravenous infusion of Dextran given if there were signs of circulatory failure

In most of the experiments the synaptic actions were investigated by conditioning monosynaptic test reflexes from posterior biceps semitendinosus gastrocnemius soleus and tibialis anterior+extensor digitorum longus (deep peroneal) To evoke monosynaptic reflexes double stimuli were often given the strength of the first stimulus was subliminal for discharge of the motoneurones but facilitated the second volley (ECCLES and LUNDBERG 1959 b) The advantage of this technique is that relatively large monosynaptic reflexes can be obtained and the size of the reflex can easily be adjusted After pontine lesions the excitability of the alpha motoneurones increases considerably and to equal the size of monosynaptic reflex evoked by double volleys in the decerebrate cerebellectomized state it was after a low pontine lesion often sufficient to use single submaximal Ia stimulation In most of the experiments the monosynaptic reflexes were recorded from the ventral roots but sometimes recording was made from the peripheral muscle nerve If so the ventral roots were intact and the reflex evoked by a single volley in the appropriate dorsal root

For conditioning single stimuli were given to various muscle and cutaneous nerves and to the posterior knee joint nerve *Condenser discharges (half time of decay 45 μ sec)* were used for stimulation Even at maximal strength these pulses did not activate C fibres 4—6 different conditioning strengths were generally used for each muscle nerve and the strength always measured relative to threshold strength for the nerve (*cf* ECCLES and LUNDBERG 1959 b) At each strength usually 10—15 different intervals between conditioning and testing volley were selected and 10—20 superimposed records were taken (*cf* Fig 1

In some experiments a simplified technique was applied to measure the effect of a conditioning volley by superimposing about 200 monosynaptic test reflex in one photographic exposure (*cf* Fig 7) In order to obtain a scatter of the traces on the records the test reflex was automatically delayed in successive sweeps so as to traverse the screen and for each record this procedure was repeated 4—5 times The CRO brightness of the baseline was kept very weak and brightening pulses were applied to the grid of the CRO during the discharges The super

imposed unconditioned test reflexes give a band of a certain width and the effect of a conditioning volley given at a fixed interval after the sweep start is displayed as a widening (facilitation) or narrowing (inhibition) of the band.

Intracellular recording from motoneurones was made as described by BROCK *et al.* (1952) with microelectrodes filled with 3 M KCl or 0.6 M K_2SO_4.

Brain stem lesions were made with a 3 mm wide spatula perpendicularly from the floor of the 4th ventricle and always through the entire medial brain stem. After the experiment the brain stem was fixed in 10 per cent formalin. Lesions in the spinal cord were controlled histologically as described by LAPORTE, LUNDBERG and OSCARSSON (1956 a).

Chapter I

Effects of brain stem lesions on actions by conditioning volleys in somatic afferents

The effect of conditioning volleys *in muscle, joint* and cutaneous afferents has been tested on monosynaptic reflexes from extensors and flexors after lesions at various brain stem levels

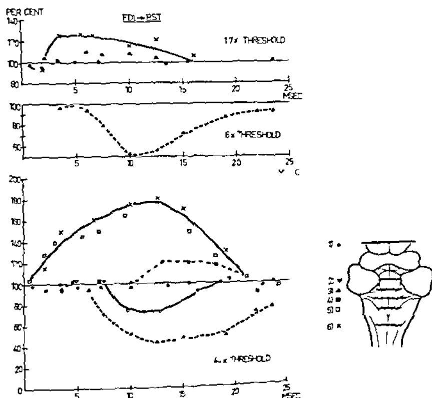


Fig 1 The effect of a conditioning volley in the nerve to flexor digitorum longus (FDL) on the monosynaptic test reflex from the nerves to posterior biceps semitendinosus (BSt) 100 % on the ordinate represents the unconditioned amplitude of the test reflex Conditioned amplitude expressed as percentage of control amplitude is plotted as a function of time interval between the incoming conditioning and testing group I volleys recorded in the dorsal root entry zone The measurements were obtained after the lesions indicated in the drawing The conditioning strengths expressed in multiples of threshold strength are indicated in each set of curves Unanaesthetized cat

A Conditioning volleys in muscles afferents

Fig 1 and 2 are from the same experiment and the curves show the effects of single conditioning volleys from the nerve to flexor digitorum longus (FDL) on monosynaptic test reflexes from the posterior biceps semitendinosus (BSt) (Fig 1) and the medial gastrocnemius (med G) (Fig 2) nerves The curves were obtained after the lesions indicated in the schematic drawing in Fig 1 In the decerebrate state lesion 1 (●) there was no effect by a conditioning volley evoked either at 1 7 times threshold which was maximal for group I or at 40 times threshold After lesion 2 (▽) there was at a conditioning strength of 40 times threshold release of inhibition to motor nuclei of extensors as well as flexors and after lesion 3 (▲) these inhibitory effects increased considerably There were no marked effects by group I volleys after this lesion but the conditioning FDL volley evoked at 6 times threshold caused inhibition of the Bst test reflex to 50 % and of the med G test reflex to 20 % (Fig 1 and 2) With lesion 4 (■) there was no significant change of the inhibition to the extensor nucleus (middle curve Fig 2) but in the flexor nucleus inhibition reversed to a small facilitation With lesion 5 (□) this facilitatory action increased and did not change with section 6 (x) below obex Inhibitory curves to the extensor nucleus were not taken after lesion 5 but after lesion 6 the inhibitory effect evoked at 6 and at 40 times threshold

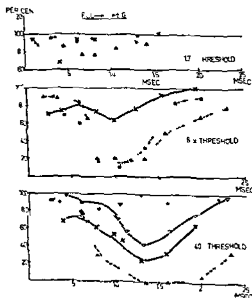


Fig 2 The curves were obtained in the same experiment as those of Fig 1 but the monosynaptic test reflex was evoked from the nerve to medial gastrocnemius (med G)

Effects of brain stem lesions on actions by conditioning volleys in somatic afferents

The effect of conditioning volleys in muscle, joint and cutaneous afferents has been tested on monosynaptic reflexes from extensors and flexors after lesions at various brain stem levels.

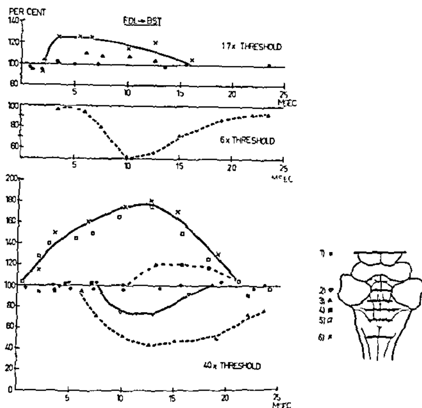


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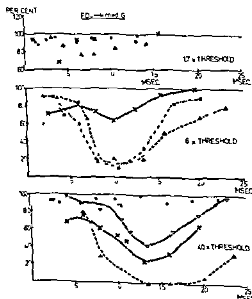


Fig 2 The curves were obtained in the same experiment as those of Fig 1 but the monosynaptic test reflex was evoked from the nerve to medial gastrocnemius (med G)

was smaller than after lesion 3 and 4. Such a decrease was usually not observed (*cf* Fig. 4) and was possibly due to a deterioration in the preparation such as may easily occur after transection of the cord. Our impression is that the pontine lesion at which there is maximal inhibitory action to flexor motor nuclei there is concomitantly maximal or nearly maximal release of the inhibitory path from high threshold muscle afferents to extensor motor nuclei. In the experiments of Fig. 1—4 the lesions were restricted to the medial part of the brain stem but no difference was found when the lesion was extended laterally so that the whole brain stem was transected.

The interneurones mediating group Ib actions are also tonically inhibited from centres in the brain stem in the decerebrate cat (ECCLES and LUNDBERG 1959 b) and the release of this control was also investigated after various lesions in the brain stem. The effects in the upper curves of Fig. 1 and 2 are difficult to judge but the findings in Fig. 4 are representative for experiments in which there was a clear Ib inhibitory action in the spinal state. The upper plot in Fig. 4 shows that there was no significant release of the group I path from FDL to gastrocnemius soleus (G S) after lesion 3 (▲), which caused an opening of the inhibitory path from high threshold muscle afferents to flexor motoneurones (Fig. 3 lower curve ▲) and concomitantly an almost maximal release of the inhibitory path to extensors from these afferents (Fig. 4 lower curve ▲). Apparently medullary centres suffice to maintain the tonic inhibitory control of interneurones of the Ib inhibitory path whereas pontine centres are required for the tonic control

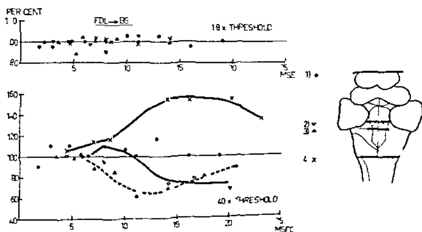


Fig. 3. As in Fig. 1 showing the conditioning effect in the nerve to FDL on the monosynaptic test reflex evoked from the BSt nerve

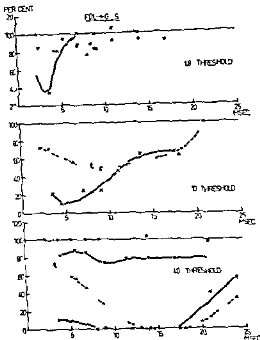


Fig 4 These curves were obtained in the same experiment as those of Fig 3 and show the effect on monosynaptic test reflexes evoked from the gastrocnemius soleus (G S) nerve

of the inhibitory path to extensor nuclei from high threshold muscle afferents Evidence for a differential supraspinal control of the Ib *inhibitory* path and of the inhibitory path from the FRA to extensor motoneurones has been presented by ECCLES and LUNDBERG (1959 b) In the experiment of Fig 3 and 4 there was no evidence of a group Ib excitatory action to flexor motoneurones after section of the spinal cord (upper curve Fig 3) Such an effect is found in Fig 5 where in the spinal state the facilitatory effect on BSt was evoked by conditioning the G S nerve at a maximal group I strength of 1 7 times threshold This effect was caused by impulses in Ib afferents because it appeared at a strength of 1 3 times threshold and when the strength was raised above 1 7 there was no increment in facilitation until the strength was raised to 2 0 times threshold A release of the Ib excitatory pathway from the tonic inhibitory control was found only with lesions caudal to the one opening the inhibitory path from high threshold muscle afferents to flexor motoneurones

There was never any evidence that Ib impulses could evoke inhibitory action in flexor motoneurones after the low pontine lesion In the experiment of Fig 5 a recording was made in the S 1 ventral root and the conditioning G S volley gave a monosynaptic discharge as large as the testing

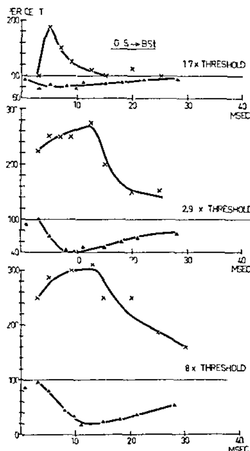


Fig. 5. As in Fig. 1 but the conditioning volley was in the nerve from gastrocnemius soleus (G.S.)

one from BSt. The small inhibitory action by the group I volley from G.S. in the upper curve (Fig. 5) was probably neither a Ib effect nor an effect due to stimulation of low threshold group II fibres but presumably a Renshaw effect secondary to the monosynaptic discharge from G.S. This can be stated because the inhibitory effect appeared at a stimulus strength only slightly above threshold for the monosynaptic reflex from the G.S. nerve. It became maximal at a stimulus strength of 1.3 times threshold, at which strength G.S. monosynaptic reflex was maximal and it did not increase when the stimulus strength was raised to activate fibres of the high threshold group I range (*cf.* ECCLES, ECCLES and LUNDBERG 1957 a).

The curves of Fig. 5 are of particular interest because of the large effects caused by impulses in low threshold group II fibres, which in the nerve from gastrocnemius soleus have been identified as spindle afferents with flower spray endings (HUNT 1954). In the decerebrate state (no curves shown in Fig. 5) conditioning at a strength of 40 times threshold had no effect. After the low pontine lesion (▲) there is inhibition to 40 % at a conditioning strength of 2.9 times threshold and at 8 times threshold the

effect is considerably increased At 16 times threshold (not shown in the Fig) the test reflex was completely inhibited A maximal group I volley was evoked at 1 7 times threshold and there was no increment from the inhibition shown in the upper curve in Fig 5 until the conditioning stimulus strength was raised above 2 0 times threshold Hence inhibition may be evoked by very low threshold group II afferents and we assume that spindle afferents with flower spray endings are responsible for this effect In the spinal state after lesion 3 (x) there was a release of facilitation both at group I and II strength When the conditioning stimulus strength was raised above 1 7 (maximum for group I) an increment in facilitation appeared at 2 0 and in the middle curve at 2 9 times threshold The facilitatory effect was very large The facilitation increased somewhat both in magnitude and duration when the conditioning strength was raised from 2 9 to 8 times threshold

In a number of the experiments group II volleys had *no effect on test* reflexes from flexors after the low pontine lesion but an inhibitory effect appeared when the stimulus strength was raised to activate group III afferents Similar findings have been made with the excitatory path to flexor and the inhibitory to extensor motoneurones after partial release from the supraspinal control and presumably *indicate low transmitt ability in these reflex* paths Lack of group II effects is sometimes also observed in the spinal state (ECCLES and LUNDBERG 1959 a)

After a low pontine lesion volleys in high threshold muscle *afferents* were found to inhibit monosynaptic *reflexes from all flexors* investigated This is illustrated for gracilis (Grac) in Fig 6 and 12 for deep peroneal (DP) *in Fig* 7 and for iliopsoas (Ip) in Fig 8 Also the effects on test reflexes from posterior biceps semitendinosus extensor digitorum longus and tibialis anterior were separately investigated all of them were inhibited by volleys in high threshold muscle *afferents from* extensors in the low potine preparation This *inhibition* could be evoked from all *extensors investigated* quadriceps (Q) FDL, G S and plantaris (Pl) The inhibitory effects were often more marked to DP than to the other *flexors* In the low potine preparation high threshold volleys *from* nerves to flexor muscles are also effective inhibitors to monosynaptic reflexes from flexors as illustrated in *Fig 6 in* which the test reflex from Grac was conditioned by volleys from the DP nerve To the left are shown *the* effects *after the* low pontine lesion the *conditioning* strength being 1 4 (just maximal for group I) 3 2 9 0 *and* 45 times threshold After transection of the cord (curves to the right) there was the usual reversal to

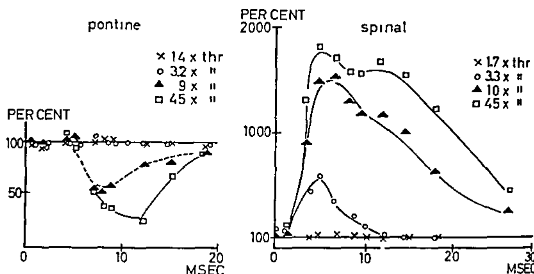


Fig 6 As in Fig 1 but the conditioning volley was in the nerves to extensor digitorum longus and tibialis anterior (DP) and the monosynaptic test reflex was evoked from the nerves to gracilis (Grac) and semitendinosus The left curves were obtained after a low pontine lesion and the right curves after spinal transection in L_1 The curves were obtained at different strengths of conditioning stimulation and given in multiples of threshold strength

facilitation Inhibition in the low pontine preparation was found with all combinations of conditioning and testing between BSt DP and Grac

The low pontine lesion not only releases some inhibitory reflex arcs but also increases the excitability of alpha motoneurones In the experiment of Fig 7 the DP test reflex was conditioned from FDL at just maximal group I strength (middle row) and at 58 times threshold (lower row) The records in each column were taken after the lesions indicated in the headings The height of the DP test reflex was adjusted after the various lesions but the changes in the alpha excitability is reflected by the size of the monosynaptic reflex discharge evoked by the conditioning volley (solid black spike in the two lower rows of records) After a low pontine lesion the monosynaptic reflex from FDL increases enormously (I and I) Records G—I were taken after section of the ventral quadrants in Th 10 The inhibitory action from high threshold afferents is maintained after this lesion (*cf* Holmqvist and Lundberg 1959 a) but the monosynaptic reflex from FDL has decreased to about the same size as in B and C This shows that the high alpha excitability in the low pontine state depends on ventral pathways Release to a spinal state after section of the remaining dorsal quadrants is shown in K—L It was a regular finding that the

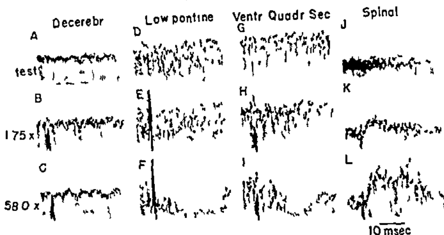


Fig The records consist of superimposed *traces of many monosynaptic reflex dis charges* evoked from the nerve to DP and recorded in the L_7 ventral root The records were obtained in the decerebrate state (A—C) after a low pontine lesion (D—F) after transection of the ventral quadrants in Th_{10} (G—I) *and after complete spinal tran section* (J—L) The unconditioned test discharges are shown in A D G and J The effect of a conditioning volley in the nerve to FDL is shown in the two lower rows of records and the stimulus strengths *expressed in multiples of threshold strength for* the nerve are indicated to the left of each row The strength of 1 75 times threshold was just maximal for group I The solid black spike in the beginning of records B C E F H I K and L is the *monosynaptic discharge in L_7 evoked by the conditioning* volley in FDL All records were taken at the same amplification Some of the records are retouched

monosynaptic reflexes increased after the low pontine lesion and this happened not only with reflexes from extensors but to a similar extent with the monosynaptic reflexes from nerves to flexors The pronounced alpha rigidity *(cf* GRANIT 1955 TERZUOLO and TERZIAN 1953) *obtained* by anaemic decerebration (POLLOCK and DAVIS 1923 1927 1930) where by half the cerebellum and a considerable part of pons is destroyed is probably due mainly to destruction of pontine structures

It is known that I a fibres from certain flexors particularly BSt make monosynaptic connexions with extensors *(anterior biceps and semimem branosus)* (ECCLES and LUNDBERG 1958) It is therefore possible that a testing volley from such a flexor may excite extensor as well as flexor motoneurones to discharge into the recording ventral root This is espe cially likely after a low pontine lesion which enhances the excitability of alpha motoneurones (Fig 7) Thus when after a low pontine lesion

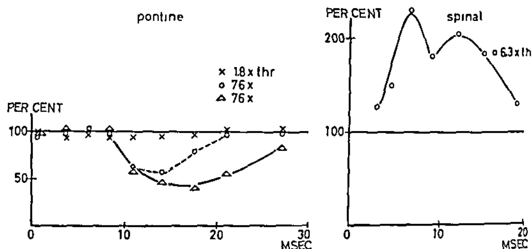


Fig. 8. As in Fig. 1 but conditioning volley in the nerve to FDL and the monosynaptic test reflex recorded in the nerve to iliopsoas (Ip) and evoked on stimulation of the dorsal 5th lumbar root. In this experiment the 4th, 5th and 6th lumbar dorsal roots were cut close to the dorsal root ganglion but the ventral roots of these segments were intact. In the 7th lumbar and 1st sacral segments the dorsal roots were intact but the ventral roots cut. The left curves were obtained after a low pontine lesion and the right curve after a spinal transection in Th12. The conditioning stimulus strengths are given in multiples of threshold strength. A just maximal group I volley from FDL was evoked at a strength of 1.8 times threshold.

the monosynaptic ventral root discharge is reduced by a conditioning volley in high threshold muscle afferents this reduction could be due to the inhibitory removal of some fraction of the ventral root spike contributed by extensor motoneurones alone.

To show that flexor motoneurones were in fact inhibited, monosynaptic reflexes were recorded peripherally in the nerves to Ip, Grac., semitendinosus (St) and DP. With the former two nerves monosynaptic reflexes were evoked by single stimuli to the sectioned L5 and L6 dorsal roots; in both cases these test reflexes could be inhibited by volleys in high threshold muscle afferents as is illustrated for Ip in Fig. 8. When testing St or DP the ventral and dorsal roots were intact and the stimulating and recording electrodes were on the muscle nerve; the test stimulus was limited to a part of the Ia volley and was subthreshold for the alpha efferents. Conditioning volleys from other muscle nerves inhibited these test reflexes effectively in the low pontine state and when the stimulus strength was raised above 5 times threshold there was a large increment in inhibition that could not have been caused by Renshaw inhibition from antidromic impulses in alpha afferents but must be ascribed to impulses in high threshold afferents.

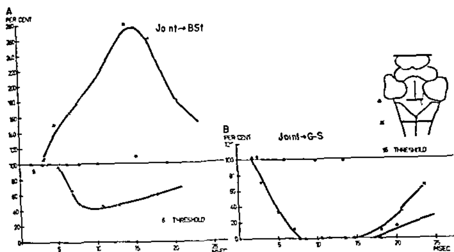


Fig 9 As in F g 1 but cond t on ng volley in the poster or nerve from the knee joint (Jo nt) The effect on monosynapt c test reflexes from BSt are shown in A and on test reflexes from gastrocnem us soleus (G) are shown in B

B Cond tioning volleys in high threshold joint afferents and in skin afferents

High threshold joint afferents and skin afferents act synergically with high threshold muscle afferents in a varity of neuronal systems (OSCARSSON 1957 1958 ECCLES and LUNDBERG 1959 a HOLMQVIST et al 1960) and it will now be shown that after a low pontine lesion volleys in these afferents evoke similar effects as a volley in high threshold muscle afferents The curves in Fig 9 A show the effects of a volley from the posterior nerve to the knee joint on the BSt monosynaptic reflex In the decerebrate state this conditioning volley had no effect (●) but after a low pontine lesion which also released inhibitory action from high threshold muscle afferents the same volley gave rise to a marked inhibition (▲) After a section below obex (x) there was a reversal to facilitation the characteristic effect in the spinal preparation The inhibitory action in the low pontine state like the excitatory in the spinal (*cf* ECCLES and LUNDBERG 1959 a) only appeared when the stimulus strength was raised above 2—3 times threshold presumably it is caused by fibres in the prominent group of 7—? μ (SKOGLUND 1956) The curves in Fig 9 B show that in the decerebrate state there was no action from the joint nerve on an extensor test reflex (●) but that a release occurred after a low pontine lesion (▲) after spinal section the inhibition decreased somewhat in duration (x)

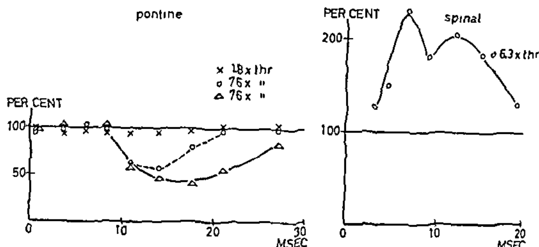


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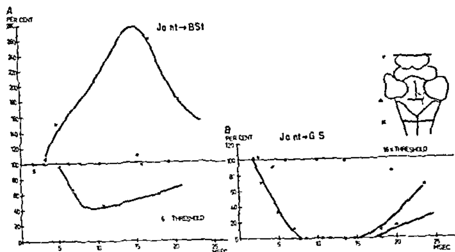


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B Conditioning volleys in high threshold joint afferents and in skin afferents

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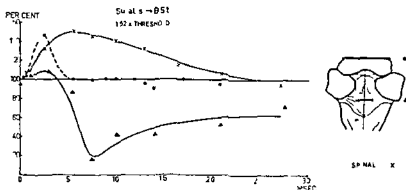


Fig 10 As in Fig 1 but conditioning volley in the sural nerve The spinal transection was made in Th_{12}

Actions by volleys in cutaneous afferents were also compared in the *decerebrate decerebellectomized, in the low pontine and in the spinal* states We have confirmed the observation by ECCLES and LUNDBERG (1959 b) that in the decerebrate state the cutaneous effects are less regularly as completely suppressed as those from high threshold muscle afferents There are sometimes in the decerebrate state marked facilitatory actions to flexor and inhibitory to extensor test reflex In other experi

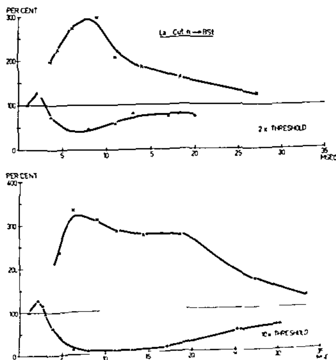


Fig 11 As in Fig 1 but conditioning volley in the lateral cutaneous nerve

ments there are only small effects in the decerebrate state from the sural or the lateral cutaneous nerve usually a short lasting facilitation to both BSt and G S After a low pontine lesion volleys in cutaneous nerves caused predominantly inhibition but there was regularly some initial facilitation To obtain the curves in Fig 10 the monosynaptic test refelex from BSt was conditioned with volleys from the sural nerve There was a brief initial facilitation in the decerebrate state (●) which after a low pontine lesion (▲) was reduced and followed by a prevailing inhibition which reversed to facilitation (×) after spinal transection With stronger stimulation very marked effects were sometimes found as in Fig 11 where the inhibition was almost complete on stimulation at 10 times threshold of the lateral cutaneous nerve Hence it appears that also cutaneous volleys evoke marked inhibitory effects in the low pontine preparation The effects on test reflexes from G S and FDL were tested in the same experiments and it was found that the low pontine lesion releases the inhibitory path from cutaneous afferents to extensor motoneurones in the same way as has already been illustrated for the effects from high threshold muscle afferents and high threshold joint afferents

Summary

The release of actions to alpha motoneurones by volleys in the FRA (group II and III muscle afferents high threshold joint and skin afferents) and in I b afferents has been investigated after various brain stem lesions in unanaesthetized decerebrate cats

A low pontine lesion released inhibition from the FRA to extensors as well as flexors The inhibitory actions were found in all flexor nuclei tested (iliopsoas gracilis semitendinosus posterior biceps extensor digitorum longus and tibialis anterior)

Release of excitatory action from the FRA to flexor nuclei was found only after a more caudal lesion in the medulla

The inhibitory and excitatory actions to flexors were evoked from the same afferent nerves

Release of excitatory and inhibitory actions by I b volleys from extensors did not occur after a low pontine lesion but only after a more caudal medullary lesion

The low pontine lesion produced a marked increase of the excitability

ised excitability

Chapter II

Effects of brain stem lesions on actions evoked by natural stimulation of receptors

To test the functional significance of the inhibitory actions on flexor nuclei described in chapter I experiments with natural stimulation of muscle and skin have been made after a low pontine lesion A comparison *of these effects has been made with the actions evoked in the decerebrate* and spinal states

A Muscle receptors

PAINTAL (1960) and BESSOU and LAPORTE (1960 1961) have shown that most of the group III afferents are activated by pressure on the muscle PAINTAL (1961) has found that this type of stimulation gives the same effect in motoneurones as a group III volley evoked by electrical stimulation of the nerve Since the inhibitory pathway to flexor moto neurones is effectively activated by group III volleys pressure of muscle has been tried in the low pontine preparation not only to study the func tional significance of the inhibitory pathway from the FRA to flexor moto neurones but also to investigate whether it could be activated by the natu ral stimuli which give excitation in the spinal state In these experiments it was important to exclude that an effect of muscle pressure could be *due to impulses in group Ia afferents which almost unavoidably* are co activated Pressure was applied to the gastrocnemius soleus plantaris muscles hence DP could not be used for testing because of the reciprocal *Ia inhibition* Neither was it possible to use BSt since its motor nucleus has the same segmental distribution as those of the above muscles and an inhibitory effect could have been caused by Renshaw inhibition sec ondary to monosynaptic Ia activation of ankle extensor motoneurones To avoid these complications monosynaptic reflexes from the nerve to Ip (segments L4—L5) or Grac (segments L5—L6) were used for testing When the gracilis nerve was used recording was made either from the fifth lumbar *or from the rostral* half of the 6th lumbar ventral root The motor nuclei to gastrocnemius soleus plantaris are in L7 and S1 and

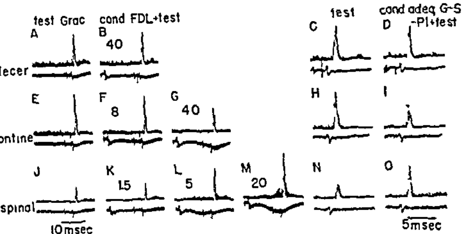


Fig 12 The effects of a conditioning volley in the nerve to FDL (B F G K L M) and of natural stimulation of the gastrocnemius soleus plantaris muscles (D I and O) on the monosynaptic test reflex from the nerves to gracilis and semitendinosus (shown uncond tioned in the upper traces in each record in A E J and C, H N) The L_6 ventral root was split in two approximately equal parts and recording was made from the rostral filament Lower traces in each record are triphasic record ngs of the incoming volley from the dorsal root entry zone In records D I and O the muscles were squeezed about 1 cm rostral to the musculotendinous region The records were obtained in the decerebrate state (A D) after a low pont ne lesion (E—I) and after spinal transection in L_1 (J—O) The condition ng stimulus strengths indicated in the records are expressed in multiples of threshold strength Each record consists of 5 superimposed traces

Renshaw inhibition does not extend beyond half a segment (ECCLES FATT and KOKETSU 1954) hence these combinations could be used The Ip monosynaptic test reflex was recorded (with a buried electrode) from the nerve to this muscle on stimulation of the 5th lumbar dorsal root Fig 12 shows the effect of muscle pressure on the Grac test reflex in the decerebrate state (C and D) after a low pontine lesion (H and I) and in the spinal state (N and O) For comparison the effect of conditioning FDL volleys is shown in the corresponding records to the left In the decerebrate state neither a strong conditioning volley at 40 times threshold nor firm squeezing of the muscles had any effect After the pontine lesion the conditioning volleys at 8 times threshold which is nearly maximal for group II (ECCLES and LUNDBERG 1959 a) had hardly any effect in record F but when the strength was raised to 40 times threshold to include also group III there was a marked inhibition (G) and pressure of the muscles also inhibited the test reflex (I) There was no inhibition when the muscles were stretched After transection of the cord (Th 12) the same conditioning

Chapter II

Effects of brain stem lesions on actions evoked by natural stimulation of receptors

To test the functional significance of the inhibitory actions on flexor nuclei described in chapter I experiments with natural stimulation of muscle and skin have been made after a low pontine lesion. A comparison of these effects has been made with the actions evoked in the decerebrate and spinal states.

A. Muscle receptors

PAINTAL (1960) and BESSOU and LAPORTE (1960, 1961) have shown that most of the group III afferents are activated by pressure on the muscle. PAINTAL (1961) has found that this type of stimulation gives the same effect in motoneurones as a group III volley evoked by electrical stimulation of the nerve. Since the inhibitory pathway to flexor motoneurones is effectively activated by group III volleys, pressure of muscle has been tried in the low pontine preparation not only to study the functional significance of the inhibitory pathway from the FRA to flexor motoneurones but also to investigate whether it could be activated by the natural stimuli which give excitation in the spinal state. In these experiments it was important to exclude that an effect of muscle pressure could be due to impulses in group Ia afferents which almost unavoidably are co-activated. Pressure was applied to the gastrocnemius-soleus-plantaris muscles, hence DP could not be used for testing because of the reciprocal Ia inhibition. Neither was it possible to use BSt since its motor nucleus has the same segmental distribution as those of the above muscles and an inhibitory effect could have been caused by Renshaw inhibition secondary to monosynaptic Ia activation of ankle extensor motoneurones. To avoid these complications monosynaptic reflexes from the nerve to Ip (segments L4—L5) or Grac (segments L5—L6) were used for testing. When the gracilis nerve was used, recording was made either from the fifth lumbar or from the rostral half of the 6th lumbar ventral root. The motor nuclei to gastrocnemius-soleus-plantaris are in L7 and S1 and

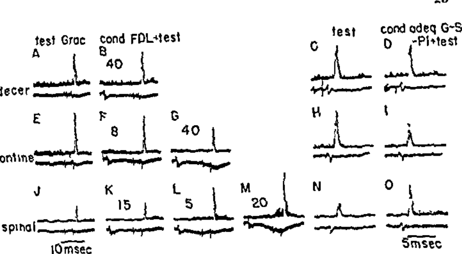


Fig 12 The effects of a conditioning volley in the nerve to FDL (B F G K L M) and of natural stimulation of the gastrocnemius soleus plantaris muscles (D I and O) on the monosynaptic test reflex from the nerves to gracilis and semitendinosus (shown unconditioned in the upper traces in each record in A E J and C H N) The L_6 ventral root was split in two approximately equal parts and recording was made from the rostral filament Lower traces in each record are triphasic recordings of the incoming volley from the dorsal root entry zone In records D I and O the muscles were squeezed about 1 cm rostral to the musculotendinous region The records were obtained in the decerebrate state (A—D) after a low pontine lesion (E—I) and after spinal transection in L_1 J—O) The conditioning stimulus strengths indicated in the records, are expressed in multiples of threshold strength Each record consists of 5 superimposed traces

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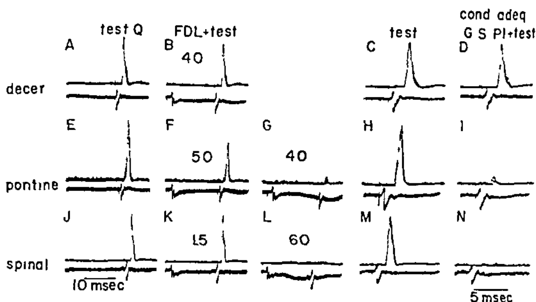


Fig 13 These records were obtained in the same experiment as those in Fig 12 but the testing monosynaptic reflex was from the nerve to quadriceps (Q)

volley evoked reflex discharges in the ventral root and facilitated the Grac test reflexes, the increment in facilitation from L to M presumably can be attributed largely to group III fibres Pressure on the muscle facilitated markedly the test reflex and this effect could be attributed mainly to fibres smaller than group II since strong stretch of the muscle (> 500 g) increased the Grac test reflex by less than 20 per cent

The corresponding records in Fig 13 are from the same experiment as Fig 12 but the monosynaptic test reflex was evoked from the Q nerve Likewise squeezing of gastrocnemius soleus plantaris had no effect in the decerebrate state (D) After the pontine lesion there was profound inhibition (I) and after section of the cord complete inhibition (N)

Bessou and Laporte (1960) have described 3 types of group III muscle afferents 1) slowly adapting fibres activated by relatively strong compression of the muscle close to the tendon 2) rapidly adapting fibres activated by localized pressure on the peroneal insertion of soleus 3) fibres activated by moderate pressure on a limited region of the muscle We have tested stimuli of these 3 types in a variety of preparations In the spinal state flexor nuclei are facilitated and extensors inhibited by all these three kinds of stimuli and in addition by a very gentle pressure on the tendon These effects must have been due largely to fibres smaller than group II because the effects obtained with strong pull of the muscle were much smaller Compression of the muscle was the most effective stimulus

but very marked effects were also obtained on touch of the muscle belly or of the tendon In excitable spinal preparation a very gentle pressure (on a surface of approximately 25 mm²) on the muscle belly or on the tendon was sufficient to evoke a clear cut effect

In the low pontine preparation test reflexes from G S were inhibited by all three types of natural stimuli described by BESSOU and LAPORTE and in addition by gentle pressure on the tendon Inhibition of test reflexes from flexors on the other hand was usually found only on compression of the muscle close to the tendon Only in one experiment did a gentle pressure on the muscle give a slight inhibition Undoubtedly further experiments are required but our tentative explanation is that the difference in natural stimulation required for evoking excitation and inhibition respectively in flexor motoneurones is quantitative This difference may be due to a weak linkage to the latter pathway in the low pontine state and can not at present be taken to indicate that afferents with different receptive functions supply these actions

B Skin receptors

HAGBARTH (1952) has shown that the cutaneous effects to flexor as well as to extensor motoneurones are complex The modality of action depends on the skin area stimulated Hence for the further analysis of the cutaneous effects in the low pontine preparation it was desirable to use natural stimulation of skin In these experiments the muscles (except some of the hip) were denervated The effect of pinching of the skin was examined on the monosynaptic reflex evoked from BSt and in one control experiment also on the test reflexes from DP and Grac In the spinal preparation the pattern of excitatory and inhibitory skin areas described by HAGBARTH (1952) was usually confirmed but in the decerebrate decerebellectomized animal there was no predictable pattern In some experiments as in Fig 14 there was no facilitation of BSt monosynaptic reflexes even on strong pinching of any area of skin of the hindlimb but a slight inhibition was obtained from the skin (pinching) over the heel (records A and B Fig 14) After a low pontine lesion the same stimulation of the skin gave strong inhibition (records C and D) and inhibition was obtained from a wide skin area as is shown in the drawing After transection of the cord there was facilitation from the skin over the heel (records E and F) as well as from other skin areas but in accordance with HAGBARTH's scheme inhibition from the skin field overlying quadriceps

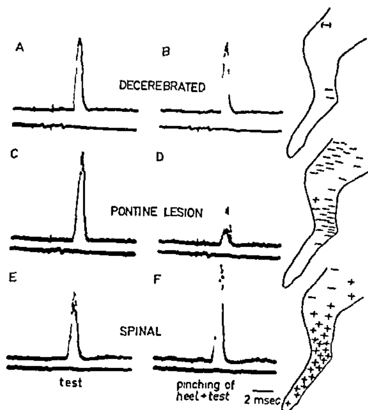


Fig 14 Effect of natural stimulation of the skin (pinching) on the monosynaptic test reflex from BSt, shown unconditioned in A, C and E (upper traces) Lower traces are triphasic recordings from the dorsal root entry zone The muscles of the hind limb were denervated except some at the hip the tibial nerve was cut Records B D and F were obtained during pinching of the skin over the heel The records were obtained in the decerebrate state (A and B) after a low pontine lesion (C and D) and after a spinal transection (E and F) In the corresponding diagrams are shown the receptive skin fields for the excitatory (+) and the inhibitory (—) effects Brackets denote weak or uncertain effect Corresponding effects were found on the medial and lateral side of the limb

Exceptionally there were marked inhibitory effects to BSt in the decerebrated state as is illustrated to the left in Fig 15 A with the reversal to facilitation in the spinal state to the right In other animals the effect from skin in the decerebrate state resembled the one found in the spinal state This is illustrated in Fig 15 B where inhibition nevertheless dominated after the low pontine lesion In Fig 15 C the test reflex was from DP It was important to do this control experiment because with test reflexes from BSt it could not be excluded that in the low pontine state a small part of the monosynaptic test discharge was derived from hip extensor motoneurones (*cf* p 17) In the experiment of Fig 15 C no effect was found

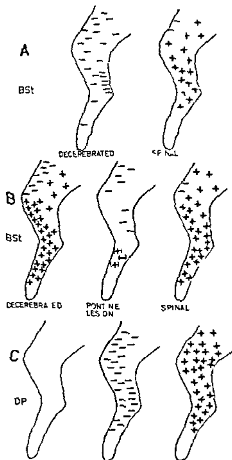


Fig. 15. As in Fig. 14 the diagrams represent the receptive skin fields for excitatory and inhibitory effects evoked by pinching the skin in three experiments A, B and C. In A and B the monosynaptic test reflex was from the BSt and in C from the DP nerve.

in the decerebrate state on pinching the skin anywhere on the ipsilateral hindlimb but after a low pontine lesion there were strong inhibitory effects from the entire hindlimb on pinching. Also pressure of the skin gave some inhibitory effects in this experiment, whereas in all other experiments pinching was required. In this experiment there was facilitation on light pressure after cord transection; in some of the other experiments marked reactions were evoked in the spinal state only when the skin was pinched.

Summary

The effect of natural stimulation of muscle and skin receptors on monosynaptic test reflexes was investigated in the decerebrate preparation, after a low pontine lesion and after spinal transection.

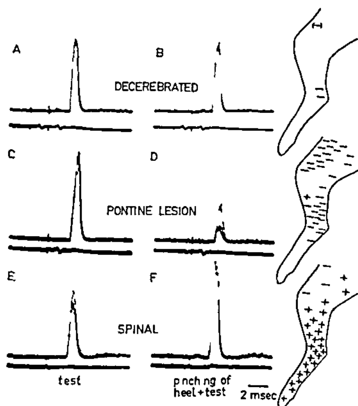


Fig. 14. Effect of natural stimulation of the skin (pinching) on the monosynaptic test reflex from BSt shown unconditioned in A, C and E (upper traces). Lower traces are triphasic recordings from the dorsal root entry zone. The muscles of the hind limb were denervated except some at the hip; the tibial nerve was cut. Records B, D and F were obtained during pinching of the skin over the heel. The records were obtained in the decerebrate state (A and B), after a low pontine lesion (C and D) and after a spinal transection (E and F). In the corresponding diagrams are shown the receptive skin fields for the excitatory (+) and the inhibitory (—) effects. Brackets denote weak or uncertain effect. Corresponding effects were found on the medial and lateral side of the limb.

Exceptionally there were marked inhibitory effects to BSt in the decerebrated state as is illustrated to the left in Fig. 15A with the reversal to facilitation in the spinal state to the right. In other animals the effect from skin in the decerebrate state resembled the one found in the spinal state. This is illustrated in Fig. 15B where inhibition nevertheless dominated after the low pontine lesion. In Fig. 15C the test reflex was from DP. It was important to do this control experiment because with test reflexes from BSt it could not be excluded that in the low pontine state a small part of the monosynaptic test discharge was derived from hip extensor moto neurones (*cf.* p. 17). In the experiment of Fig. 15C no effect was found

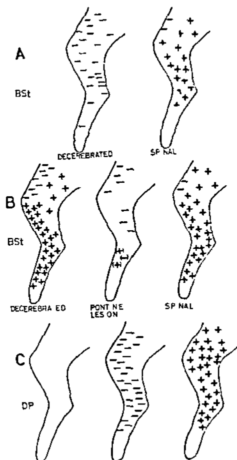


Fig 15 As in Fig 14 the diagrams represent the receptive skin fields for excitatory and inhibitory effects evoked by pinching the skin in three experiments A B and C In A and B the monosynaptic test reflex was from the BSt and in C from the DP nerve

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Summary

The effect of natural stimulation of muscle and skin receptors on monosynaptic test reflexes was investigated in the decerebrate preparation after a low pontine lesion and after spinal transection

Manipulation of a muscle (pressure) designed to activate group III afferents had no effect in the decerebrate state but inhibited test reflexes from flexors and extensors after a low pontine lesion After a spinal transection there was facilitation of flexor and inhibition of extensor test reflexes

In the decerebrate state stimulation of the skin (pinching or pressure) varied in different preparations with respect to the effect on monosynaptic test reflexes from flexors but in some cases there was no or little effect After a low pontine lesion there was inhibition, and after spinal transection facilitation of flexor test reflexes from a major part of the skin areas over the ipsilateral hindlimb

Chapter III

Analysis of the inhibitory path from the FRA to flexor motoneurones

The low pontine preparation offers better possibilities for analysis of the inhibitory path to flexor motoneurones than either the spinal preparation (ECCLES and LUNDBERG 1959 a) or the anaesthetized preparation with intact brain (*cf* Chapter IV). In the latter types of preparation absence of excitatory actions from the FRA is found only occasionally and for each motoneurone it must be excluded that inhibitory effects are not RENSHAW effects secondary to discharges evoked in other flexor motoneurones by volleys in the FRA. In the low pontine state single volleys in the FRA do not excite either flexor or extensor motoneurones and the inhibitory path can be investigated without the complications mentioned above.

A Intracellular recording from flexor motoneurones after a low pontine lesion

Intracellular recording was made from DP and BSt motoneurones *in* 5 cats with a low pontine lesion. Volleys in the FRA were found *to* evoke inhibitory post synaptic potentials (IPSPs) in flexor motoneurones as is illustrated in Fig. 16 and 17. The intracellular records in *Fig.* 16 *are from* a DP motoneurone and were obtained with a K_2SO_4 *electrode*. The effect from the Q nerve in C and D evoked at 3.7 and 7.4 times threshold can be ascribed to impulses *in group II afferents* while inhibitory actions from other nerves FDL (E) BSt (*H*) Pl and G S (not illustrated) appeared mainly at group III strengths. Volleys *in the* sural nerve (I and J) also give a large IPSP. The joint nerve was not dissected for stimulation in this experiment. In C and D the onset of the IPSP starts 3.2 msec after the incoming group I volley. Considering the *conduction* velocity of group II fibres this suggests a central *latency of* 2.8 msec and indicates that the inhibitory path has two *interneurones*; the same holds true for the path from cutaneous afferents as indicated by *the* latencies in I and J.

Since the ventral roots were sectioned antidromic activation from muscle nerves could not be used; motoneurones had to be identified from their pattern of I a convergence (*ECCLES et al* 1957 b, ECCLES and LUND

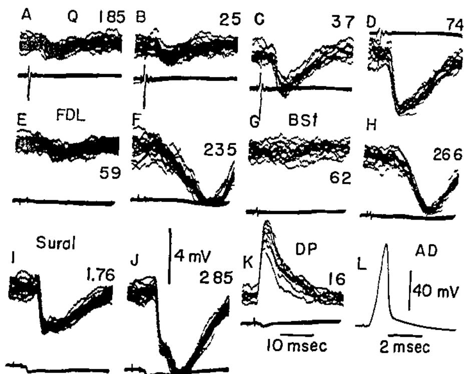


Fig 16 Intracellular recording (upper traces except in D) with microelectrode filled with 0 6 M K_2SO_4 from a motoneurone of the DP nerve Lower traces (except in D) are triphasic recordings from the L_6 dorsal root entry zone The effects were evoked on stimulation of the nerves to quadriceps (Q) (A—D) FDL (E and F) BSt (G and H) DP (K) of the sural nerve (I and J) and of antidromic activation (AD) from the L_7 ventral root (L) The stimulus strengths expressed in multiples of threshold strength are indicated in the records The animal was decerebrated and decerebellectomized and had a low pontine lesion with the approximate location of lesion 3 in Fig 1 The records consist of many superimposed traces

BERG 1958) Cells with monosynaptic Ia EPSP from BSt were only accepted as BSt motoneurones if they did not receive monosynaptic Ia excitation from the hip extensors anterior biceps and semitendinosus (*cf* ECCLES and LUNDBERG 1958) In Fig 17 recording (KCl electrode) was made from a BSt cell after a low pontine lesion The effects were evoked by volleys in the Q nerve (A—H) and in the G S nerve (I—P) In order to keep the IPSPs in the hyperpolarizing direction a depolarizing current was passed in A—D and I—L The reversed IPSPs obtained during passage of a weak hyperpolarizing current are seen in E—H and M—P The reciprocal Ia IPSP is seen in A and E but the later effects in B—D and F—H were evoked by volleys in high threshold muscle afferents As in Fig 16 the effects from high threshold afferents in the Q nerve appeared at lower stimulus strengths and after a shorter latency than the effects

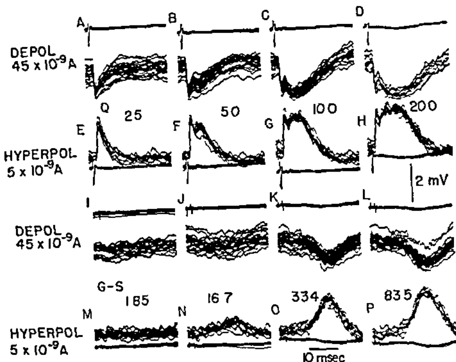


Fig 17 Intracellular recordings (lower traces in A—D I—L upper traces in E—H and M—P) with micro electrode filled with 3 M KCl from a motoneurone of the BSt nerve A—H responses evoked by stimulation of the nerve to quadriceps Q and I—P of the nerve to gastrocnemius soleus (G S) A—D and I—L were obtained during passage of a depolarizing current through the recording electrode and L—H and M—P during passage of a hyperpolarizing current The triphasic records of the incoming volley were recorded from the L_6 dorsal root entry zone in A—H and M—P but from L_7 dorsal root entry zone in I—L The records consist of many superimposed traces

from the other nerves There was no sign of a concealed FPSP Effects by volleys in homonymous high threshold afferents could not be analyzed in the cells of Fig 17 since the cell was discharged by the Ia volley from the BSt nerve but in other cells it was established that IPSPs were evoked from these afferents as well (*cf* Fig 21 and also Fig 8 ECCLES and LUNDBERG 1959 a)

B Exclusion of Wilson's disinhibition

When we discovered that after a low pontine lesion volleys in the FRA inhibited flexor test reflexes we took it for granted (HOLMQVIST and LUNDBERG 1959 b) that this was due to a release of the inhibitory path

was already recognized in the spinal state (ECCLES and LUNDBERG 1959a). However, the investigations by WILSON and his collaborators makes it necessary to consider another neuronal route for the IPSPs found in flexor motoneurones after a low pontine lesion. WILSON (1959) confirmed RENSHAW's (1941, 1946) finding that impulses in recurrent collaterals from alpha afferents may facilitate motoneurones and found that the latency for this facilitation may be brief. The effect was most common from extensors to flexors (WILSON, TALBOT and DIECKE 1960a) and is most probably caused by disinhibition (WILSON, DIECKE and TALBOT 1960b). The low pontine lesion increases the alpha motoneurone excitability enormously; almost invariably there is a resting alpha discharge in this state. The low pontine lesion also releases the inhibitory path from the FRA to extensor motoneurones. Hence a volley in the FRA may give cessation of alpha activity in extensor motoneurones leading to removal of disinhibition to flexor motoneurones, *i.e.* the establishment of an IPSP in flexor motoneurones. Because of the prolonged transmitter action in Renshaw cells (ECCLES, FATT and KOKETSU 1954, ECCLES, ECCLES, IGGO and LUNDBERG 1961) we would not expect an abrupt onset of the IPSP evoked via such a route and the short latency IPSPs from Q in Figs. 16 and 17 cannot be explained in this way. However, volleys in high threshold afferents from Q sometimes evoke IPSPs in flexor motoneurones also in the decerebrate state (Fig. 4, ECCLES and LUNDBERG 1959b, HOLMQVIST and LUNDBERG 1959a).

That there is a true release of the inhibitory path from the FRA to flexor motoneurones has been proved as illustrated in Fig. 18. Monosynaptic reflexes were recorded from the nerves to medial vastus (med V) (A—C, K—M, U—W) and Grac (F—H, P—R, Z—BB) (stimulation of the cut 6th lumbar dorsal root). In the decerebrate state supramaximal volleys in the G S nerve had no action on these test reflexes (not illustrated). After a low pontine lesion there was inhibition of both the extensor and flexor test reflex (B, C and G, H respectively). Recording at higher gain revealed resting activity (presumably in alpha afferents) in the nerve to med V, D, but not in the nerve to Grac, I. A conditioning volley in high threshold afferents from G S inhibited this resting activity, E, but had no effect in record J. Thereafter the ventral quadrants were sectioned in L 1. There was a marked decrease in the sizes of monosynaptic reflexes (*cf.* Fig. 7); the dorsal root stimulus was raised in order to evoke a med V test reflex and to evoke a Grac test reflex the dorsal root stimulus had to be supported by a double volley from the semitendinosus nerve (*cf.* ECCLES, ECCLES and LUNDBERG 1957b). *At the same time the resting*

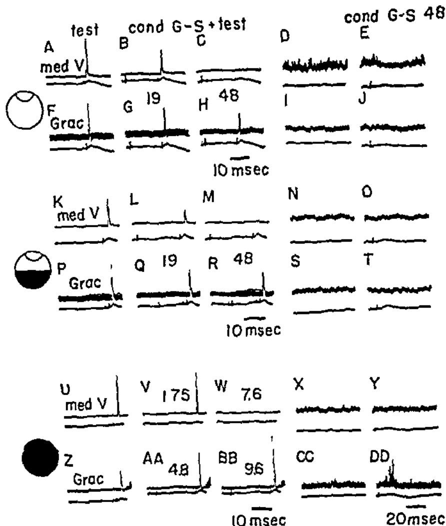


Fig 18 In the left column is shown the unconditioned monosynaptic test reflexes recorded in the nerves to medial vastus (med V) (A, K and U) evoked on *stimulation* of the L_4 dorsal root and to gracilis (Grac) (F, P and Z) evoked on stimulation of ...

... with stimulation of the gastrocnemius soleus nerve The experiment was made on decerebrate cerebellectomized animal with a low pontine lesion with the approximate location of lesion 3 *in Fig 1* As indicated by the symbols to the the left these records were obtained with the spinal cord intact (A—J), after section of ... of the cord in ... superimposed

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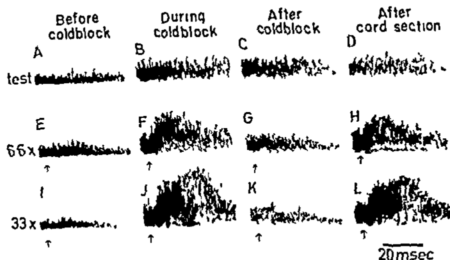


Fig 19 As in Fig 7 the records consist of superimposed traces of many reflex discharges evoked from the nerve to BSt In A—D are shown the unconditioned monosynaptic reflexes in E—L the effect of a conditioning volley in the nerve to FDL Arrows indicate the time of arrival at the dorsal root entry zone of the group I volley from the FDL nerve Conditioning stimulus strengths are given as multiples of threshold strength for the nerve Records A E and I were obtained in a decerebrate cat [illegible] Th_{12} so a thermode could be [illegible] and J were obtained during [illegible] mode temperature 0°C) and D H and L after transection in Th_{10} of the remaining parts of the cord

show the unconditioned test reflex from BSt During the coldblock there is the same release of facilitation (F and J) evoked by a conditioning FDL volley as after transection of the remaining dorsal parts of the spinal cord (H and L) Though the coldblock in B F and J lasted for 45 min the recovery was complete in less than 5 min the action of the FDL volley is equally well suppressed after the coldblock (C G and K) as it was before (A E and J)

Summary

Intracellular recording was made from motoneurones in decerebrate unanaesthetized cats with a low pontine lesion Volleys in the FRA evoked large IPSPs in flexor motoneurones and there was no evidence of concealed EPSPs Latency measurements suggest that the shortest inhibitory path to flexor motoneurones has two interneurones

discharge in the nerve to med V ceased (record N) The descending paths tonically inhibiting the interneurones of the spinal arcs are located in dorsal part of the lateral funicle (HOLMQVIST and LUNDBERG 1959 a) and after section of the ventral quadrants there was no release of excitation to Grac (P) and the inhibition from G S to the extensor was not significantly changed (L and M) Furthermore, and this is the crucial point *the inhibitory effect from G-S to Grac remained* (records Q and R) Hence *the inhibition of flexor motoneurones after the low pontine lesion can* not to any significant extent be due to inhibition of extensor motoneurones giving removal of WILSON's disinhibition and the pontine lesion must have released a 'genuine inhibitory path to flexor motoneurones Release to the spinal state is shown in U—DD There is facilitation of the Grac test reflex (AA and BB) and at high conditioning strength also discharge in Grac motoneurones (DD)

C. The relationship between the excitatory and the inhibitory path from the FRA to flexor motoneurones

It will be shown in the discussion concluding this paper that the inhibitory and excitatory action evoked from the FRA in flexor motoneurones in all likelihood are evoked by impulses in the same afferent fibres which have two alternative pathways to flexor motoneurones as was tentatively suggested by ECCLES *and* LUNDBERG *(1959 a) The interrelationship between these two paths with oppo*site action offers an interesting problem In spinal cats ECCLES and LUNDBERG failed to obtain evidence that the EPSP evoked by volleys in high threshold *afferents conceal an IPSP This could indicate a reciprocal relationship between* the excitatory and inhibitory paths to flexor motoneurones but in order to prove this it would be necessary to record intracellularly from the same flexor motoneurones first under conditions of supraspinal control favouring the inhibitory path from the FRA and then in the spinal state favouring the excitatory path

It is possible to remove the inhibitory supraspinal control of interneurones reversibly by cold blockage of the dorsal half of the cord (HOLMQVIST *et al* 1960) We have tried extensively to utilize the cold block technique on animals with low pontine lesions in conjunction with intracellular recording but these experiments failed presumably due to fragility of the pontine preparation there was circulatory shock and collapse of the supraspinal control from the brain stem The cold block technique is exemplified in Fig 19 The experiment was done on a decerebrate cat About 15 mm of the ventral quadrants were removed in the lower thoracic region and a thermode inserted under the dorsal cord In *our previous experiments* (HOLMQVIST *et al* 1960) the dorsal column was removed at the site of the block but now we leave the dorsal column intact thereby improving circulation in the cord over the thermode with the assurance of better reversibility With a thermode temperature of 0°C it was not possible to block all dorsal column fibres but by functional tests the blockage of the more ventrally located descending pathways responsible for the supraspinal control of reflex arcs was complete This is *illustrated in* Fig 19 where the upper records

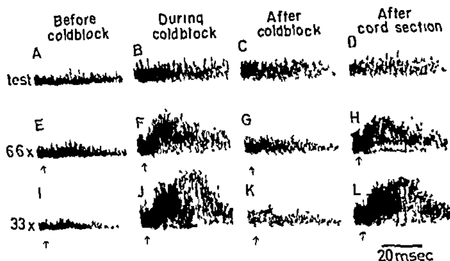


Fig 19 As in Fig 7 the records consist of superimposed traces of many reflex discharges evoked from the nerve to BSt In A—D are shown the unconditioned monosynaptic reflexes in F—L the effect of a conditioning volley in the *nerve to FDL* Arrows indicate the time of arrival at the dorsal root entry zone of the group I volley *from the FDL nerve* Conditioning stimulus strengths are given as multiples of threshold strength for the nerve Records A E and I were obtained in a *decerebrate cat* with the ventral quadrants removed bilaterally in Th_{10}—Th_{12} so a thermode could be inserted under the remaining dorsal parts of the *cord* B F and J were obtained during and C G and K after cold block of the dorsal cord (thermode temperature 0°C) and D H and L after transection in Th_{10} of the remaining parts of the cord

show the unconditioned test reflex from BSt During the coldblock there is the same release of facilitation (F and J) evoked by a conditioning FDL volley as after transection of the remaining dorsal parts of the spinal cord (H and L) Though the coldblock in B F and J lasted for 45 min the recovery was complete in less than 5 min the action of the FDL volley is equally well suppressed after the coldblock (C G and K) as it was before (A, E and J)

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Inhibition of flexors after the low pontine lesion remained after cessation of the resting activity in extensor motoneurones following section of the ventral quadrants Hence the inhibition of flexors is not secondary to inhibition of extensor motoneurones causing cessation of a resting discharge in them leading to removal of facilitation in the flexor motoneurones (WILSON's disinhibition) It is concluded that the low pontine lesion has released a 'genuine' inhibitory path from the FRA to flexor motoneurones

An improved method is described for reversible blockage of descending spinal pathways

Chapter IV

Reflex actions in the anaesthetized animal with intact brain

HOLMQVIST and LUNDBERG (1959 a) compared the effects of volleys in high threshold muscle afferents on animals with intact brain after decerebration and in the spinal state. The supraspinal inhibitory control of interneurones of spinal reflex arcs was less intense when the brain was intact than after decerebration. Although smaller, the effects when the brain was intact resembled those found after section of the cord. In further experiments on animals with intact brain lightly anaesthetized with Nembutal we have now found that volleys in the FRA in some animals can give considerable inhibition of flexors as is illustrated in Fig. 20. Record A shows the unconditioned monosynaptic BSt test reflex and B—E the effects of conditioning volleys from G S evoked at 2.1 (maximal for group I), at 5.2, at 10.5 and 21 times threshold. Inhibition of DP was found more frequently than of BSt but in this experiment the DP test reflex was facilitated. Since other flexor nuclei were excited it is difficult to exclude entirely that the inhibition is not a recurrent Renshaw effect (RENSHAW

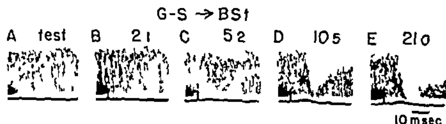


Fig. 20. As in Fig. 7 the records consist of superimposed traces of many monosynaptic reflex discharges but they were evoked from the nerve to BS[illegible] in S_1. The [illegible] volleys from [illegible] strengths re[illegible] [illegible] in the records. The animal was anaesthetized with Nembutal and the brain was intact. The lower trace is the base line which was not completely suppressed in these recordings. The monosynaptic reflex evoked by the conditioning volley emerges from the baseline and serves as time reference for the incoming volley

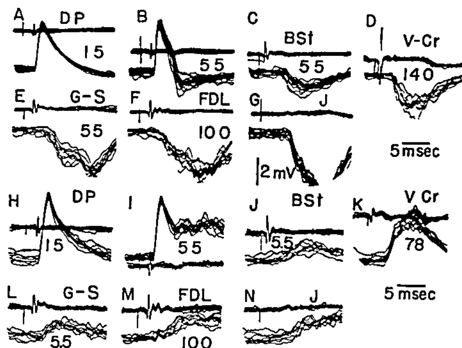


Fig 21 Lower traces are intracellular recordings with a micro electrode filled with 0 6 M K_2SO_4 from two DP motoneurones A—G and H—N respectively The upper traces in the records were recorded from the dorsal root entry zone The brain of the animal was intact and A—G were obtained before and H—N after transection of the spinal cord in L_1 The following nerves were stimulated DP (A B and H I) BSt (C and J) vastocrureus (V Cr) (D and K) gastrocnemius soleus (G S) (E and L) FDL (F and M) and the posterior nerve to the knee joint (J) (G and N) The stimulus strengths relative to threshold strength are indicated in the records

1941 1946 ECCLES *et al* 1954) the base line in D and L actually reveals that the volley in high threshold afferents from G S gave a late discharge in the ventral root which can be seen as a slight elevation of the basline following the G S monosynaptic spike

Intracellular recording from motoneurones was also made in lightly anaesthetized cats with intact brain The intracellular records in Fig 21 are from two DP motoneurones A—G were obtained before and H—N from another motoneurone after transection of the cord In the former state IPSPs were evoked by volleys in high threshold afferents (B—G) while in the spinal state these volleys gave EPSPs (I—N) It should be noted that volleys from extensors and flexors are equally effective in giving these two sets of actions In the experiment of Fig 21 inhibition dominated in all of the 6 DP motoneurones recorded from before transection of the cord 4 DP motoneurones were found after transection of the cord and in all of these volleys in high threshold afferents evoked EPSPs BSt motoneurones investigated in the same experiment received

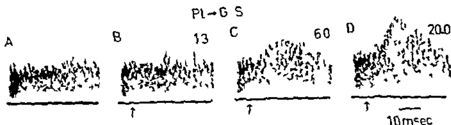


Fig 22 As in Fig 7 but conditioning volleys in the plantaris nerve (Pl) and monosynaptic test discharges evoked from the gastrocnemius soleus nerve The brain was intact and the cat lightly anaesthetized (Nembutal) Arrows indicate the time of arrival at the dorsal root entry zone of the group I volley from the plantaris nerve Conditioning stimulus strengths are expressed in multiples of the threshold strength a maximal group I volley was evoked at 1 3 times threshold

excitatory action from these afferents also before transection of the cord The inhibitory effects in the DP motoneurone of Fig 21 were not a secondary Renshaw inhibition caused by the discharge in other flexor motoneurones because these IPSPs were evoked at stimulus strengths lower than those required for reflex discharge from the FRA in the ventral root used for recording as well as in the adjacent roots Stimulation at 10/sec was used to depress reflex excitation

In the spinal state extensor motoneurones receive predominantly inhibitory actions from the FRA There is however apart from the extensor thrust considerable evidence of ipsilateral extension reflexes from skin (GRAHAM BROWN 1911 1912 GRAHAM BROWN and SHERRINGTON 1912 HAGBARTH 1952) ECCLES and LUNDBERG (1959 a) found that volleys in high threshold muscle afferents occasionally could evoke EPSPs in extensor motoneurones The records in Fig 22 are from an experiment on a lightly anaesthetized animal with intact brain in which volleys in high threshold afferents from Pl caused considerable facilitation of G S monosynaptic test reflexes There was also a corresponding facilitation of G S from the nerves to interior biceps and BSt On the other hand in the same experiment test reflexes from Q were inhibited by volleys in high threshold afferents from G S Pl and FDL Even if the findings in this experiment are exceptional they may like the intracellular finding be indicative of an excitatory path to extensor motoneurones from high threshold muscle afferents

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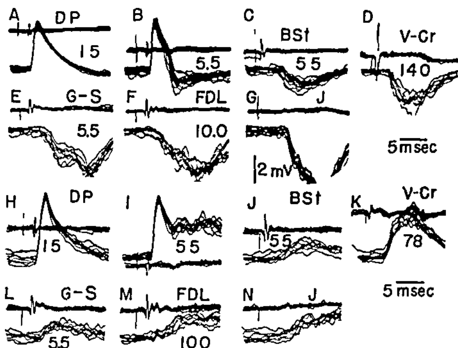


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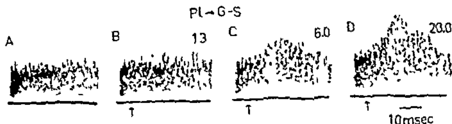


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Discussion

This investigation has been concerned with the supraspinal control of spinal reflex arcs activated by Ib afferents and of those activated by the *flexion reflex afferents (FRA) but the discussion will be confined to the* control of the latter pathways.

The supraspinal inhibitory control of the interneurones of the flexion reflex arcs was originally revealed by a comparison of the effect by single volleys in the FRA in decerebrate and spinal cats (JOB 1953, ECCLES and LUNDBERG 1959 b). The present experiments with natural stimulation of muscle and skin in the decerebrate and spinal states supplement these findings and illustrate well the effectiveness of this inhibitory control.

New aspects on the organization of the inhibitory supraspinal control of the spinal reflex actions by the FRA have been gained through the *experiments in which the release from this control was investigated after* lesions at various brain stem levels. *After a low pontine lesion there is release of the inhibitory pathway to extensor motoneurones whereas release of the excitatory path to flexor motoneurones occurs only after a more caudal medullary lesion* (Fig. 1—4). Since the low pontine lesion gives an almost complete release of the inhibitory path to extensor motoneurones without any release of the excitatory path to flexor motoneurones it is unlikely that the effect is due merely *to a quantitative difference*. A more reasonable explanation is a rostral location of the cells giving rise to the descending tract inhibiting the inhibitory path to extensor motoneurones in relation to the cells giving rise to the tract inhibiting the excitatory path to flexor motoneurones. It is however possible that the release is due to interference with centres controlling the activity of these descending tracts rather than with the neurones of these descending tracts. In any case the differential *effect of the* lesions strongly suggests that separate descending neuronal systems are responsible for the inhibitory control of the reciprocal paths from the FRA to extensor and flexor motoneurones. Hence there is also the possibility of a differential functional control of these reflex paths and that reciprocal innervation of flexor and

motoneurones is not a necessary consequence of impulses in the primary FRA afferents

A major part of this investigation has been concerned with an *inhibitory path from the FRA to flexor motoneurones*. Evidence for such an inhibitory path was originally obtained in spinal animals mainly when the blood pressure was low leading to disappearance of the normally prevalent excitatory actions from the FRA (ECCLES and LUNDBERG 1959 a). These inhibitory actions were more common in DP motoneurones but were observed also in other flexor motoneurones. In unanaesthetized high spinal animals PAINTAL (1961) also frequently found inhibition of DP (but not of BSt) monosynaptic reflexes by group III volleys from gastrocnemius soleus. In addition we have now found marked inhibitory actions to flexor motoneurones in anaesthetized animals with intact brain (Fig. 20 and 21) though usually the excitatory effects dominate (*cf*. HOLMQVIST and LUNDBERG 1959 a). In the decerebrate state both the inhibitory and the excitatory path is usually closed (*cf*. however ECCLES and LUNDBERG 1959 b, HOLMQVIST and LUNDBERG 1959 a) *but a low pontine lesion releases the inhibitory path from the FRA to flexor motoneurones without release of the excitatory path*. This inhibition reached all flexor nuclei investigated (iliopsoas, gracilis, posterior biceps semitendinosus, tibialis anterior and extensor digitorum longus).

Intracellular recordings revealed that in the low pontine state volleys in the FRA evoke IPSPs in flexor motoneurones, but even so it could not without further analysis be concluded that the low pontine lesion had released the inhibitory path found in the spinal preparation (ECCLES and LUNDBERG 1959 a). The low pontine lesion also gives a very marked increase of alpha excitability (Fig. 7) with resting discharge in alpha motoneurones. Furthermore this lesion also releases the inhibitory paths from the FRA (*cf*. above) to extensor motoneurones, thus volleys *in FRA can* decrease the resting activity in extensor motoneurones. This might lead to removal of recurrent facilitation in flexor motoneurones (WILSON 1959, WILSON *et al*. 1960 a) and since recurrent facilitation is caused by disinhibition (WILSON *et al*. 1960 b) to the establishment of IPSPs *in flexor* motoneurones (*cf*. p. 31). It was however possible to exclude that after a low pontine lesion any major part of the inhibition evoked from the FRA in flexor motoneurones had this origin because the inhibition remained after the spontaneous activity in the motoneurones had ceased following a ventral section of the spinal cord with interruption of pathways tonically exciting motoneurones (Fig. 18). It is therefore concluded that the low pontine lesion has released a genuine inhibitory path from the FRA to flexor motoneurones.

The low pontine lesion also releases inhibitory paths *from the FRA to* contralateral flexor and extensor motoneurones whereas crossed excitation is released only by a more caudal lesion (HOLMQVIST 1960, 1961). It is *extremely interesting that the centres controlling the excitatory and the inhibitory paths from the FRA to motoneurones presumably are located at different levels in the brain stem.* This finding may very well be a clue to principles according to which these supraspinal centres establish their descending connexions. It should, however, be noted that this generalization only holds with respect to the control of the effects evoked by the FRA: the Ib inhibitory path is not released by the low pontine lesion but only by a more caudal lesion. For the time being we have not tried to study the *location and organization of those centres in the reticular formation* but have been concerned with the consequences of our findings for the understanding of the organization at the segmental level of the spinal cord.

The finding that volleys in the FRA can inhibit as well as excite flexor nuclei raises the question whether there may be *two types of flexor motoneurones, one group receiving excitation and the other inhibition from the FRA.* The fact that the monosynaptic reflexes from flexors may be completely inhibited by volleys in the FRA is against this explanation and experiments with intracellular recording do not support this possibility. In spinal cats inhibitory effects by these afferents are not *uncommon in flexor motoneurones, but in some animals in good condition* in which extensive sampling from various flexor nuclei was made, excitatory action was found in all flexor motoneurones (ECCLES and LUNDBERG 1959 a). Furthermore it has also been demonstrated in many motoneurones of spinal cats that convergence of excitatory and inhibitory actions from the FRA to flexor motoneurones may occur (ECCLES and LUNDBERG 1959 a) and actually is rather common (HOLMQVIST and LUNDBERG unpublished).

To account for the excitatory and the inhibitory pathway to flexor *motoneurones from the FRA two alternatives remain:*

1) *The excitatory and the inhibitory paths may be supplied by the same afferents.*
2) *The excitatory and the inhibitory paths to flexor motoneurones may be supplied by afferents of different receptive function.*

Of these two alternatives we are for circumstantial reasons decidedly in favour of the former. Our main reason for this view stems from the similarity in the receptive fields from which the inhibitory and excitatory actions to flexor *motoneurones* are drawn. Either effect can be evoked from all the systems comprising the FRA: skin afferents, high threshold

joint afferents and high threshold muscle afferents. In particular it should be noted that for both actions there is the same peculiar convergence from extensors and flexors. With the second of the two alternatives discussed we would have to assume two sets of receptor systems from skin, muscle and joint with afferents in the same diameter ranges and both of them supplying actions to flexor motoneurones from the same extensive fields. Hence it seems much more reasonable to postulate that the same afferent fibres have two pathways to flexor motoneurones and though the evidence is entirely circumstantial in nature, it is in our opinion strong enough virtually to exclude the second alternative.

This postulate has been developed from our concept that actions by the FRA should be recognized as a pattern with functional units. This concept is based not only on the fact that these afferent systems all evoke similar effects in motoneurones but also on the finding that actions from these systems converge to the neurones of no less than 4 ascending spinal pathways (LAPORTE, LUNDBERG and OSCARSSON 1956 b; HOLMQVIST, LUNDBERG and OSCARSSON 1956; OSCARSSON 1957, 1958; LUNDBERG and OSCARSSON 1960, 1961). As for the motoneurones the receptive fields of the neurones belonging to these pathways are very large with convergence from high threshold muscle afferents of extensors and flexors. Transmission to these ascending pathways is controlled from supraspinal centres in a very similar way as the flexion reflex actions (ECCLES and LUNDBERG 1959 b; HOLMQVIST and LUNDBERG 1959 a; HOLMQVIST *et al.* 1960). It is not known to which extent convergence from the FRA to the motoneurones and to these ascending pathways occurs at an interneuronal level, but extensive convergence from high threshold muscle afferents and cutaneous afferents is found to many interneurones in the dorsal horn (ECCLES, FATT and LANDGREN 1956; KOLMODIN 1957; HUNT and KUNO 1959; ECCLES, ECCLES and LUNDBERG 1960).

The advantage with the term FRA is obvious, but it should be noted that actions evoked by cutaneous afferents and by high threshold muscle and joint afferents can only be ascribed to the FRA if they are evoked by all these afferent systems. It is possible that each of these receptor systems in addition have other specialized central pathways.

In a preliminary report of this work attention was drawn to the effects by volleys in low threshold group II fibres after various lesions in the brain stem (HOLMQVIST and LUNDBERG 1959 b). Group II fibres from gastrocnemius soleus have quantitatively been identified as spindle afferents with flower spray endings (HUNT 1954). Among the FRA low threshold group II volleys from this muscle, which in the spinal state evoke excitatory action in *flexor nuclei*, can give pronounced inhibitory action after a low pontine lesion (Fig. [illegible]). Since there is no reason to assume two subgroups of spindle afferents with flower spray endings it was inferred that the same fibres have one excitatory

Recently PAINTAL (1960)

group II muscle afferents [illegible] claims that in the group II range there is a

The low pontine lesion also releases inhibitory paths from the FRA to contralateral flexor and extensor motoneurones whereas crossed excitation is released only *by a more caudal lesion* (HOLMQVIST *1960, 1961*). It is extremely interesting that the centres controlling the excitatory and the inhibitory paths from the FRA to motoneurones presumably are located at different levels in the brain stem. This finding may very well be a clue to principles according to which these supraspinal centres establish their descending connexions. It should, however, be noted that this generalization only holds with respect to the control of the effects evoked by the FRA, the Ib *inhibitory path is not released by the low pontine lesion* but only by a more caudal lesion. For the time being we have not tried to study the location and organization of those centres in the reticular formation but have been concerned with the consequences of our findings for the understanding of the organization at the segmental level of the spinal cord.

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This postulate has been developed from our concept that actions by the FRA should be recognized as a pattern with functional unity This concept is based not only on the fact that these afferent systems all evoke similar effects in motoneurones but also on the finding that actions from these systems converge to the neurones of no less than 4 ascending spinal pathways (LAPORTE LUNDBERG and OSCARSSON 1956 b HOLMQVIST LUNDBERG and OSCARSSON 1956 OSCARSSON 1957 1958 LUNDBERG and OSCARSSON 1960 1961) As for the motoneurones the ra [illegible]

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To account for the excitatory and the inhibitory pathways to flexor motoneurones from the FRA two alternatives remain:

1) *The excitatory and the inhibitory paths may be supplied by the same afferents.*
2) *The excitatory and the inhibitory paths to flexor motoneurones may be supplied by afferents of different receptive function.*

Of these two alternatives we are for circumstantial reasons decidedly in favour of the former. Our main reason for this view stems from the similarity in the receptive fields from which the inhibitory and excitatory actions to flexor motoneurones are drawn. Either effect can be evoked from all the systems comprising the FRA: skin afferents, high threshold

of concealed IPSPs (ECCLES and LUNDBERG 1959 a) This may indicate a reciprocal linkage between the excitatory and inhibitory paths to flexor motoneurones which would be functionally meaningful but in order to decide this question finally the synaptic actions should be analysed in the same motoneurones first under conditions of supraspinal control giving optimal opening of the inhibitory path and then in the spinal state The technique of reversible blockage of the descending controlling pathways (HOLMQVIST et al 1960) was tried for this purpose (Fig 19) but so far our attempts to perform the experiment have failed

It is also possible that there are alternative pathways from the FRA to ipsilateral extensor motor nuclei There are several reports on ipsilateral extensor reflexes from skin (SHERRINGTON and SOWTON 1911 GRAHAM BROWN 1911 1912 GRAHAM BROWN and SHERRINGTON 1912 HAGBARTH 1952) but before ascribing these reflexes evoked on stimulation of cutaneous nerves to the FRA it should be excluded that they represent more specialized reflexes KUGELBERG EKLUND and GRIMBY (1960) have recently studied nociceptive spinal reflexes in man and found a harmonious integration of flexion and extension reflexes designed to defend the limb with maintenance of posture The inhibitory path from the FRA to extensor motor nuclei is very dominant in spinal cats but intracellular recordings have sometimes revealed excitatory action evoked by volleys in high threshold muscle afferents (ECCLES and LUNDBERG 1959 a) and one example of substantial facilitation of an extensor nucleus by volleys in high threshold muscle afferents was reported above (Fig 22) It is difficult to assess these findings at present but it may be worth while to look for excitatory actions from the FRA to extensor motoneurones under different conditions of supraspinal control In this connexion it is of great interest that volleys in the FRA on the contralateral side can evoke either excitatory or inhibitory action in both flexor and extensor motoneurones HOLMQVIST 1960 1961)

Our results give new aspects on the functional significance of the reflex actions from the FRA Following the disclosure of the strong inhibitory supraspinal control of the interneurones mediating the actions from FRA it was suggested that these reciprocal actions more or less could be switched off so as to permit optimal regulation of movement by the Ia and Ib systems (ECCLES and LUNDBERG 1959 b LUNDBERG 1959) For this suggestion which still remains a reasonable possibility it was an inherent assumption that the reciprocal reflex actions to flexors and extensors as expressed by SHERRINGTON (1906) were part and parcel of one

significant number of pressure pain afferents of the kind which dominate the group III range PAINTAL has not given a quantitative evaluation of the 'contamination' of group II and it is therefore difficult to appreciate the significance of his claim These pressure pain afferents were, however, found mainly in the high threshold group II range and presumably the above argument will retain its validity for low threshold group II actions

It is also important to consider inhibitory and excitatory effects in flexor motoneurones by natural stimuli known to activate the FRA PAINTAL'S (1961) *experiments with natural stimulation of muscles are of interest* in this context He found that pressure on the lateral gastrocnemius soleus muscle inhibited or facilitated DP monosynaptic reflexes in accordance *with the modality of effect evoked by single volleys in group III* afferents from the lateral gastrocnemius soleus nerve The present comparison of *the effect of natural stimulation* of muscle or skin in the decerebrate *pontine and spinal states gives a powerful illustration of the flexibility* imposed by the supraspinal control of spinal reflex arcs Natural stimulation of muscle, chosen for activation of high threshold afferents (PAINTAL *1960*, BESSOU *and* LAPORTE *1960, 1961) inhibits flexor nuclei in the low* pontine preparation (Fig 12) and likewise on adequate stimulation of skin inhibition is provided from a field which may comprise the entire *hindlimb (Fig 15) After transection of the cord the same kind of stimuli* gives facilitation from muscle and skin with the exception of the expected inhibition from the skin over the antagonist muscle (*cf* HAGBARTH 1952) *Natural activation of joints have not been employed since the receptor* function of the numerous joint afferents in the range (7—2 μ) which belong to the FRA is unknown *In summary*, natural stimuli known to *activate the FRA have been tested with respect to their ability to inhibit* or facilitate flexor motoneurones but so far there is no evidence that the inhibitory and excitatory paths require different natural stimuli Further *experimentation along* this line with more differentiated stimuli will certainly be needed when we acquire more detailed informations about receptors connected with the FRA

Further investigations are needed to disclose the functional *relationship between the excitatory and the inhibitory paths from the FRA to flexor motoneurones* Although inhibitory action from high threshold muscle afferents to flexor motoneurones are rather common in the spinal cat and convergence of excitatory and inhibitory action is found frequently (*cf* above) it should be noted that in a number of flexor motoneurones with large EPSPs from high threshold muscle afferents *there was no evidence*

6 It is further postulated that the excitatory and inhibitory actions from the FRA to flexor motoneurones are evoked by impulses in the same afferent fibres and that supraspinal centres can select either path for function

Acknowledgement This investigation was supported by grants from the Swedish Medical Research Council We are indebted to Mr E Eide for construction of electronic equipment Technical assistance was given by Miss Karin Ebbesson

and the same reflex reaction' A reciprocal organization of reflex actions has been found under a variety of circumstances (SHERRINGTON 1906 1910) but our findings show *that it is not a requisite result of a given* volley in the FRA It seems likely that supraspinal centres may exert a differential control of the reciprocal actions from the FRA to extensor and flexor motoneurones and hence that they are not necessarily a physio logical unity when governed by higher centres Furthermore there is the possibility that higher centres may choose between an excitatory and in hibitory channel to flexor motoneurones Meanwhile it should also be remembered that there is another supraspinal control system governing the transmittability of pathways from the FRA to motoneurones Activity in the pyramidal tract facilitates interneurones mediating the flexion reflex actions as well as interneurones of the Ib pathways and of the Ia inhi bitory pathway (LUNDBERG and VOORHOEVE 1961 a)

General Summary

1 The synaptic actions evoked through spinal reflex paths in moto neurones by *impulses in somatic afferents have been investigated under* different conditions of supraspinal control The technique of conditioning monosynaptic reflexes has been used as well as intracellular recording from motoneurones Effects of single volleys and of natural stimulation of muscle and skin have been investigated

2 In the decerebrate state the reflex actions by volleys in the FRA (flexion reflex afferents group II and III muscle afferents high threshold joint and cutaneous afferents) are suppressed due to a tonic inhibitory control *from the brain stem of the interneurones of these reflex arcs* (ECCLES and LUNDBERG) The release from this supraspinal control has been investigated after lesions at different brain stem levels

3 After a low pontine lesion there is release of the inhibitory path from the FRA to extensor motoneurones and in addition release of an *inhibitory* path from these afferents to flexor motoneurones The low pontine lesion increases the alpha excitability very markedly and this fact has to some extent complicated the analysis

4 *Release of the excitatory path to flexor motoneurones* occurs only after a more caudal lesion in the medulla

5 It is inferred that the pathways conveying the reciprocal actions of excitation to flexor and inhibition to extensor motoneurones from the FRA are controlled from the brain stem by separate neuronal systems and *suggested* that these reciprocal actions can be differentially controlled from supraspinal centres

6 It is further postulated that the excitatory and inhibitory actions from the FRA to flexor motoneurones are evoked by impulses in the same afferent fibres and that supraspinal centres can select either path for function

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BY

BÖRJE RUDEWALD

STOCKHOLM 1962

ACTA PHYSIOLOGICA SCANDINAVICA

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This work is part of a research program dealing with hemodynamic problems The studies, conducted by Porjé and the author, have been going on since 1954, continuing a program of hemodynamic investigation initiated by Porjé in 1944 Since early in 1961 active co-operation has been maintained with the British Medical Research Council for the purpose of promoting the study of various problems in this field

Docent I G Porjé was the first to excite my interest in hemodynamics and to him I owe warmest thanks for many years of friendly and fruitful co operation

To my chief, Dr Carl Sandström at the Department of Roentgenology, St Erik s Hospital, I wish to express my appreciation of his kindness in allowing me to use the facilities of his departement for the technical and medical studies

My thanks are also due to my friend Docent Åke Gidlund whose apparatus for rapid serial angiography was of paramount importance for certain phases of the work

Docent Gunnar Jonsson, chief of Roentgen Department I, Södersjukhuset, where some of the work was carried out, was kind enough to supply me with the radiological case reports and the films necessary for certain measurements It was my privilege to discuss with him and his colleagues matters of utmost interest as regards radiology of the heart and I wish to convey to him and his staff my cordial thanks

Docent Sven Akesson, Head of the Heart Clinic, Södersjukhuset, aided the investigation significantly by allowing me to examine patients from his clinic. I am greatly obligated to him

To Dr Johan Karnell Associate Chief of the Heart Clinic, I express my profound gratitude for his valuable contribution to the fulfillment of this work.

I am much indebted to Professor B J Andersson of the Royal Institute of Technology Stockholm for his elaboration of certain theoretical aspects of aortic hemodynamics of great importance to this investigation

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INTRODUCTION

... liquid ... ly flow
flow have been developed ... active
have been carried out by physicists and technologists ... t active
interest in oscillatory flow seems to emanate from research workers dealing with
... problems As respects flow measurements on intact man the introduction of
... ovascular system has been of fundamental
... the cardiac
... its by
...
values for flow cannot ...
for direct measurement of instantaneous flow in experimental ... t
present applicable to human beings

This work deals with problems relating to determination of instaneous flow and other hemodynamic data in the human ascending aorta The flow of a liquid along a tube is conditioned by the existence of a pressure difference between the two ends of the tube The relationship betw en pressure difference and flow is simple in the case of steady flow but more complex in the case of non steady flow In either case how ever it is possible in principle to determine the flow when the pressure difference (or the pressure gradient) between two points along the tube is known A measuring method based on determination of the pressure difference along the ascending aorta was evolved by Porje and Rudewald during the years 1955—1961 In 1957 the first results of studies on humans were presented and further studies carried out with the aid of improved apparatus were reported in 1959 1960 and 1961 In 1956 Fry Mallos and Casper using a similar technique reported studies on the dog aorta and have since extended their investigations to human beings (Barnett, Greenfield and Fox 1961)

The pressure gradient has been indirectly computed from the pulse pressure by equating its first derivative in respect to time with the pressure gradient multiplied by the pulse wave velocity Unfortunately the pulse wave velocity is frequency dependent and is in any case very difficult to measure The transmission of the pulse wave

in the arterial system is a highly complex phenomenon and only with direct measurement of the pressure gradient will the interference of reflections and non linearities be eliminated McDonald (1960) in discussing these problems states that the *direct measurement* of a pressure gradient is however, the *only reliable* method for the calculation of flow curves He also points out that the distortion introduced into a pressure gradient method by averaging the differential pressure over a finite interval is very small The same conclusion was arrived at (Porje and Rudewald 1957) in a study of a particular recursion formula proposed by B J Andersson The basic assumptions underlying the physical calculation of ascending aortic blood flow have been dealt with by Porje and by Porje and Rudewald in several works Valuable contributions to the theoretical aspects of the problem have been made by B J Andersson McDonald s monograph Blood Flow in Arteries (1960) provides an excellent survey of the problems relating to pulsatile flow

CHAPTER I

THEORY

The motion of blood in the ascending aorta has the character of a pulsatile flow in an elastic tube The laws of hydrodynamics can be applied, but in order to solve the equations certain restrictions must be introduced Thus it is necessary to make certain assumptions concerning the character of the flow as well as the physical properties of the aorta and the blood McDonald (1960) discusses the assumptions required for application of the Navier Stokes equation to the arteries He infers that although the simplified Navier Stokes equation used by Womersley cannot be strictly applied to arteries it may serve as a practical approximation for calculating oscillatory arterial flow This may be true for peripheral parts of the arterial system, but in the ascending aorta the inlet length problem and the elastic deformation of the vessel detract from the applicability of the Womersley equation It is, however, of particular interest to this study to apply the equations for laminar flow to the motion of blood in the ascending aorta For purposes of simplification the vessel is looked upon as a long straight rigid circular tube, outer forces being disregarded The equation of motion of a non compressible fluid is

$$(1) \qquad \frac{1}{\nu}\frac{\partial v}{\partial t} = \frac{\partial^2 v}{\partial r^2} + \frac{1}{r}\frac{\partial v}{\partial r} - \frac{1}{\mu}\frac{\partial p}{\partial x}$$

The solution to this equation has been given by several workers cited by McDonald (1960) Womersley's work, in particular, has been important for the understanding of this type of pulsatile flow Womersley points out that the character of the flow is determined by a non dimensional parameter, $\alpha = R\sqrt{\frac{\omega}{\nu}}$, where R is the radius of the tube ω the angular frequency and ν the kinematic viscosity The velocity profiles for different values of α have been calculated by several workers, e g Welander (1950) — Fig 1 — and Hale McDonald and Womersley (1955) With rising α values the axial core of the fluid tends to become unsheared The friction within the fluid occurs mainly in a thin layer contiguous to the wall For a tube of the same dimensions as a normal ascending aorta ($R = 1.5$ cm, $\omega = 2\pi$ rad/sec and

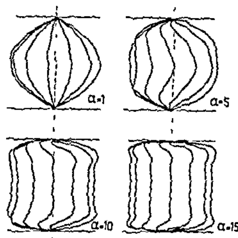


Fig 1 Velocity profiles for different α values ranging from 1—15 (From Welander, 1950)

$\nu = 3 \cdot 10^{-2}$ stokes) α will be about 20 The peripheral laminae are here considerably out of phase with the axial core, which is moving like a solid body These effects are discussed in detail by McDonald for a flow composed of several harmonics It has also been shown by Womersley (1955 b) that as α increases, the phase lag of the flow with respect to the pressure gradient tends to approach 90° and the amplitude of the flow term for a given pressure gradient becomes small as compared with that predicted by Poiseuille's law For α values of about 20 this effect should be very pronounced and the flow pattern should be dominated by the inertia of the mass of the central core For a periodic flow containing a steady flow component the pressure drop due to the steady flow can be calculated from the Poiseuille equation The pressure gradient corresponding to the oscillatory flow components will be the synthesis of the Fourier components of the pressure gradients calculated from the Womersley equation It is of particular interest to compare the amplitudes of pressure gradient and fluid velocity obtained from the general equation at high α values, with those obtained from the simplified equation valid for a non viscous flow

For this purpose an analytical approach used in acoustics by Wagner (1947) and Welander (1950) will be applied The assumption that the friction force is proportional to the fluid velocity will give a simplified equation of motion

$$\frac{\partial u}{\partial t} = - \frac{1}{\rho} \frac{\partial p}{\partial x} - \gamma u \qquad (2)$$

where γ is the constant of proportionality, which can be either real or imaginary From this equation, which can also be applied to turbulent flow, Fry *et al* (1956) derived a formula for calculation of flow in a large artery, viz the ascending aorta, from a measured pressure gradient To measure the constant Fry used an analogue

computer adjusted for zero flow at the end of diastole Fry (1959) also advanced a formula involving some tentative constants. In laminar noncompressible flow γ can be determined analytically by adjusting its value so as to derive the same flow from the simplified equation (2) as from the general equation (1) Welander (1950) puts

$$\gamma = \frac{K(\alpha) \cdot \nu}{R^2}$$

where K is a function of α In Poiseuille flow $K \simeq 8$ For the flow in a tube of the same dimensions as the ascending aorta γ would be 0 107 sec^{-1} The function $K(\alpha)$

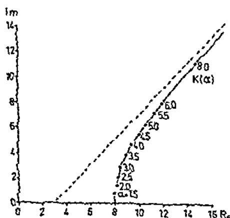


Fig 2 Real (R_e) and imaginary (I_m) parts of the function $K(\alpha)$ (From Welander, 1950)

has been tabulated by Welander (Fig 2) For high α values, K will approach an asymptotic value

$$K \simeq 2\alpha \; e^{\frac{i\pi}{4}}$$

and

$$\gamma \simeq \frac{2\sqrt{\omega \cdot \nu}}{R} \; e^{\frac{i\pi}{4}}$$

With the exponential forms of velocity and pressure gradient

$(u = u \;\; e^{i\omega t}, \; \frac{\partial p}{\partial x} = A \;\; e^{i\omega t})$ eq (2) becomes

$$-\frac{A}{\rho u} = \frac{1}{R}\sqrt{2\omega\nu} + (\omega + \frac{1}{R}\sqrt{2\omega\nu})\, i$$

Let $-\frac{A}{\rho u} = S(f)$, where f is the frequency Then the amplitude

$$|S(f)| = 2\pi f \sqrt{1+\frac{2}{R}\sqrt{\frac{\nu}{\pi f}}+\frac{4}{2\pi R^2 f}}$$

where the last term is very small compared to 1 The phase angle of $S(f)$ is given by

$$\varphi = arctg\left(1+R\sqrt{\frac{\pi f}{\nu}}\right)$$

For $\gamma = 0$, e g in non viscous flow, $S_{\gamma=0}(f) = i\omega$ The quotient

$$\left|\frac{S(f)}{S_{\gamma=0}(f)}\right| = \sqrt{1+\frac{2}{R}\sqrt{\frac{\nu}{\pi f}}} \tag{3}$$

represents for a given flow the amplitude ratio of the pressure gradient in viscous flow to that in non viscous flow For $R = 1\ 5$ cm and $\nu = 0\ 03$ stokes the ratio for frequencies ranging from 1 to 10 c/sec varies as shown in Fig 3 The amplitude in viscous flow at 1 c/sec is 6 3 % higher than in non viscous flow For higher har

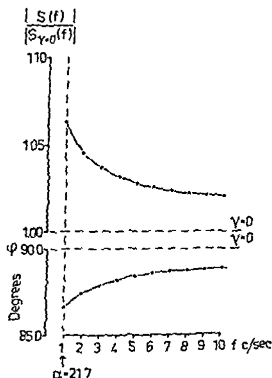


Fig 3 Amplitude and phase of the quotient defined by eq (3) for frequencies ranging from 1 to 10 c/sec The phase angle is denoted by φ See text

monics the ratio falls off but at 10 c/sec the pressure gradient is till about 2 % higher than in non viscous flow The phase angle varies as shown in Fig 3 At 1 c/sec the pressure gradient lags 3 5 degrees with respect to the corresponding non viscous pressure gradient At 10 c/sec the phase lag is about 1 degree For a given pressure gradient the relative amplitude error associated with the neglect of viscosity effects is given by

$$(4) \qquad \left|\frac{\Delta u}{u}\right| = \left|\frac{S(f)}{S_{\gamma=0}(f)}\right| - 1$$

where Δu is the amplitude difference of the velocity obtained for non viscous flow ($\gamma=0$) and that obtained from eq (2) The error will be evident from the diagram for the pressure gradient For a fundamental frequency of 1 c/sec (α about 22) the non viscous flow amplitude will be 6 3 % higher than that of viscous flow and for the tenth harmonic (α about 70) the corresponding value will be 2 %

These theoretical considerations indicate that harmonic analysis of the pressure gradient and application of the Womersley equation will be necessary for an exact calculation of the flow even for high α values This conclusion is applicable to laminar flow in a rigid tube Although its application to the ascending aortic blood flow might be defensible the character of the flow could be such as to undermine the validity of any prediction of the viscosity effects At the present time the exact nature of the blood flow in the human ascending aorta is not known and precise corrections for viscous flow in an elastic tube as given by Womersley are impracticable since they presuppose detailed knowledge of the visco elastic properties of the tissues There is good reason to suppose however that the effects of viscosity and elasticity are of a secondary order of magnitude and that the equation valid for non viscous flow will accordingly give a reasonable initial approximation for the calculation of instantaneous blood flow in the ascending aorta In a few of the cases studied in the present investigation it was possible to measure the elastic deformation of the ascend ing aorta and the relevant corrections were calculated from a previously derived formula (Porje and Rudewald 1961)

If we regard the ascending aorta as a cylindrical tube with elastic walls and without branches the solution of the equations of motion for the flow of a non viscous and incompressible fluid will be (Porje and Rudewald 1961)

$$(5) \qquad i_m(t) = Q_m(0)\ U(t) + \int_0^t \frac{2Q_m U}{Q(0\ \tau)}\ \frac{dQ_m}{d\tau}\ d\tau$$

where i_m is the mean instantaneous flow and Q_m the mean cross sectional area of the ascending aorta between $x=0$ and $x=L$ The notations used are shown in Fig 4 The fluid velocity $U(t)$ computed from the simple formula

Let $-\frac{A}{\rho u}=S(f)$, where f is the frequency. Then the amplitude

$$|S(f)|=2\pi f\sqrt{1+\frac{2}{R}\sqrt{\frac{\nu}{\pi f}}+\frac{4}{2\pi R^2 f}}$$

where the last term is very small compared to 1. The phase angle of $S(f)$ is given by

$$\varphi=\operatorname{arctg}\left(1+R\sqrt{\frac{\pi f}{\nu}}\right)$$

For $\gamma=0$, e g in non-viscous flow, $S_{\gamma=0}(f)=i\omega$. The quotient

(3)
$$\frac{|S(f)|}{|S_{\gamma=0}(f)|}=\sqrt{1+\frac{2}{R}\sqrt{\frac{\nu}{\pi f}}}$$

represents for a given flow the amplitude ratio of the pressure gradient in viscous flow to that in non viscous flow For $R=1\,5$ cm and $\nu=0\,03$ stokes the ratio for frequencies ranging from 1 to 10 c/sec varies as shown in Fig 3 The amplitude in viscous flow at 1 c/sec is 6 3 % higher than in non viscous flow For higher har

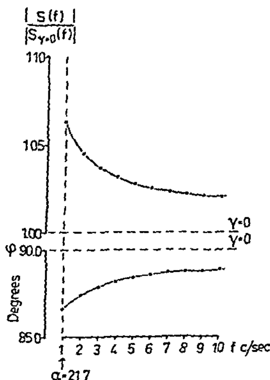


Fig 3 Amplitude and phase of the quotient defined by eq (3) for frequencies ranging from 1 to 10 c/sec The phase angle is denoted by φ See text

monics the ratio falls off, but at 10 c/sec the pressure gradient is still about 2 % higher than in non viscous flow The phase angle varies as shown in Fig 3 At 1 c/sec the pressure gradient lags 3 3 degrees with respect to the corresponding non viscous pressure gradient At 10 c/sec the phase lag is about 1 degree For a given pressure gradient the relative amplitude error associated with the neglect of viscosity effects is given by

$$(4) \qquad \left|\frac{\Delta u}{u}\right| = \left|\frac{S(f)}{S_{\gamma=0}(f)}\right| - 1$$

where Δu is the amplitude difference of the velocity obtained for non viscous flow ($\gamma = 0$) and that obtained from eq (2) The error will be evident from the diagram for the pressure gradient For a fundamental frequency of 1 c/sec (α about 22) the non viscous flow amplitude will be 6 3 % higher than that of viscous flow, and for the tenth harmonic (α about 70) the corresponding value will be 2 %

These theoretical considerations indicate that harmonic analysis of the pressure gradient and application of the Womersley equation will be necessary for an exact calculation of the flow even for high α values This conclusion is applicable to laminar flow in a rigid tube Although its application to the ascending aortic blood flow might be defensible the character of the flow could be such as to undermine the validity of any prediction of the viscosity effects At the present time the exact nature of the blood flow in the human ascending aorta is not known and precise corrections for viscous flow in an elastic tube as given by Womersley are impracticable since they presuppose detailed knowledge of the visco elastic properties of the tissues. There is good reason to suppose however that the effects of viscosity and elasticity are of a secondary order of magnitude and that the equation valid for non viscous flow will accordingly give a reasonable initial approximation for the calculation of instantaneous blood flow in the ascending aorta In a few of the cases studied in the present investigation it was possible to measure the elastic deformation of the ascending aorta and the relevant corrections were calculated from a previously derived formula (Porje and Rudewald 1961)

If we regard the ascending aorta as a cylindrical tube with elastic walls and without branches the solution of the equations of motion for the flow of a non viscous and incompressible fluid will be (Porje and Rudewald 1961)

$$(5) \qquad i_m(t) = Q_m(0)\; U(t) + \int_0^t \frac{2Q_m U}{Q(0,\tau)} \; \frac{dQ_m}{d\tau}\, d\tau$$

where i_m is the mean instantaneous flow and Q_m the mean cross sectional area of the ascending aorta between $x=0$ and $x=L$. The notations used are shown in Fig 4 The fluid velocity $U(t)$ computed from the simple formula

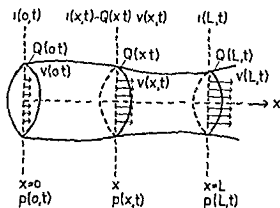


Fig 4 Diagram of an elastic tube to illustrate the notations used The coordinate of length is taken along the axis of the tube Instantaneous flow is denoted by $i(x,t)$, cross sectional area by $Q(x,t)$, fluid velocity by $v(x\ t)$ and pressure by $p(x\ t)$

$$(6)\qquad U(t)=\frac{1}{\rho L}\int_{0}^{t}\Delta p\cdot d\tau+C$$

where Δp is the differential pressure, will approximate the mean instantaneous blood velocity within a segment of the ascending aorta of length L. If it is assumed that at the end of diastole $(t=0)$ the fluid velocity is zero, then $C=0$ Due to the coronary flow, the blood is not at rest at the end of diastole even in cases with normal aortic valves, but the error introduced by this postulate is probably small except in cases with valvular insufficiency or high heart rates An advantage of formula (6) is that the function $\rho\ L\ U(t)$ can be obtained directly from the measured differential pressure by means of a simple electrical integrator If Q_m is known, the first term to the right in formula (5) can be determined The second term, representing an initial correction for the changes in area of the aorta, must be computed graphically or numerically

The volume of liquid, V_s, calculated from

$$(7)\qquad V_s=\int_{0}^{t_s} i_m\cdot dt$$

were t_s is the systolic time, will correspond to the left ventricular stroke volume minus the systolic flow to the coronary arteries

The first derivative of $U(t)$, denoted by $a(t)$

$$(8)\qquad a(t)=\frac{dU(t)}{dt}=\frac{\Delta p}{\rho L}$$

corresponds to the mean instantaneous blood acceleration within a portion of the ascending aorta of length L.

The potential and kinetic energy delivered by the heart to the aorta has heretofore been calculated from values for mean pressure and mean flow In the absence of a method for measuring instantaneous blood flow this approximation has been essential even though its limitations in humans have not been defined From the instantaneous pressure and blood flow values obtained by the method presented here a more legitimate calculation of potential energy can be made with the following formulae e g (Aperia 1940)

$$W(t)=\int_0^t p \; i_m \; d\tau \qquad \text{kinetic energy} \tag{9}$$

$$E(t)=\frac{\rho}{2}\int_0^t \frac{i^3{}_m}{Q^2{}_m} \, d\tau \qquad \text{potential energy} \tag{10}$$

CHAPTER 2

APPARATUS

Manometry

The theoretical and experimental problems connected with the design of mano meters for intravascular measurement of pulsatile pressure were studied in detail by Hansen (1949) and Hansen and Warburg (1950) Subsequently Wood and co workers (1954) and Wood (1956) among others analyzed the physical properties of manometer catheter systems with particular reference to the harmonic content of the arterial pulse pressure

The main technical problem involved in the differential pressure method of measuring instantaneous blood flow is the determination of the pressure difference between two points along the aorta In principle this value can be established by obtaining the electrical difference between the outputs of two single sided manometers — a method utilized by Barnett, Greenfield and Fox (1961) Considerable technical difficulties however, are involved in balancing statically and dynamically this type of system Alternatively, a differential manometer may be used Manometers of this kind have, until recently, been characterized by relatively poor frequency responses Porje and Rudewald were fortunate enough to have the co operation of A Soderholm and P Engstrom in developing an adequate differential manometer which has been described in a paper by Porje and Rudewald (1961)

The natural frequency of this differential manometer and a fine double lumen catheter is of the order of 25 c/sec To record faithfully a periodic pressure wave form with such a system two alternatives are available Firstly the damping can be so adjusted that the amplitude response of the system is virtually independent of the applied frequency within a given frequency range and the phase shift is proportional to frequency For a pressure wave form composed of frequencies within this range the record will be an undistorted reproduction of the wave form though it will show a time lag Secondly damping can be adjusted so that frequencies within the lower part of the frequency curve can be recorded with little distortion of amplitude and phase, and the wave form will be reproduced without a time lag The latter alternative is preferable for exact work and was the method used here The deflection of the transducer membrane changes the ohmic resistance of an unbounded strain gauge (Soderholm, 1952) arranged to form a Wheatstone bridge This is fed with a carrier

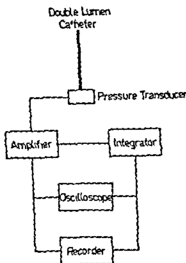


Fig 5 Block diagram of the experimental set up

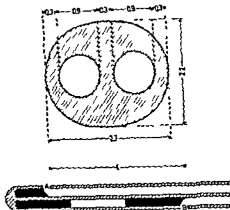


Fig 6 Transverse section of the double-lumen catheter Dimensions are given in millimeters The lower diagram is a section through the tip of the catheter Solid black indicates gold inserts The openings (A and B) are separated by distance *l*

of 1000 c/sec Amplification and demodulation are done in conventional circuits The signal is fed to the D C amplifier of an Elema 4-channel ink recorder and the input of an integrating circuit The integrator output is fed to another channel of the recorder A block diagram of the arrangement is shown in Fig 5

Catheters

In the early part of this work two single lumen catheters were used, necessitating two arterial punctures Vibrations of these catheters, however, often caused marked disturbances on the tracings It was obvious that the use of a double lumen catheter would simplify the technique and ensure greater reliability Since the available catheters were not suitable a special double-lumen catheter was designed and constructed in cooperation with *Svenska Metallverken* (Porjé and Rudewald 1961) The catheter was composed of the radiopaque material used by Ödman (1956)

The design and dimensions are shown in Fig 6 The catheter has an elliptical transverse section and its lumina are circular Suitable lengths (115 cm) are prepared in the laboratory The lateral holes, *2 to 3 mm in length, are scooped out towards* the axis of the catheter This shape is thought to reduce the chances of flow stagnation at the measuring site The proximal part of the catheter is fitted with three gold inserts to increase the mass at that end Experience suggests that catheter motions due to the impact of circulating blood are reduced by this measure The catheter is connected to the pressure transducer by the attachment shown in Fig 7.

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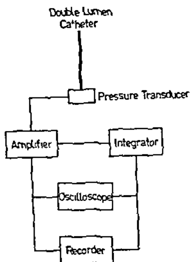


Fig 5 Block diagram of the experimental set up

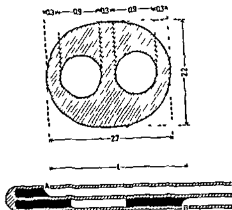


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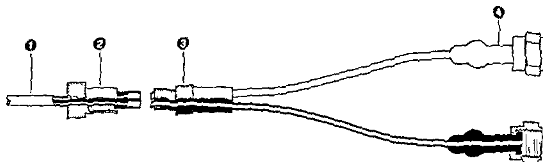


Fig 7 The catheter (1), provided with a small collar, is forced by the screw piece (2) on to the attachment (3). Screws (4) are fitted to stopcocks and the transducer

Performance of the Catheter manometer System

At the highest sensitivity of the transducer amplifier recorder system, 25 mm paper deflection was elicited by a pressure of 20 cm H_2O. The drift at this sensitivity was less than 0.5 % of full deflection per hour. Under optimal conditions for the same sensitivity range the drift at the integrator output for zero differential pressure could be adjusted to less than 1 % of full deflection, equivalent to 1 cm H_2O sec per minute

Fig 8 Amplitud response and phase lag as functions of the ratio of driving frequency (ω) to resonant frequency (ω_R) detoned by γ ($\omega_R = 26$ c/sec)

Static calibration was carried out with a mercury manometer for pressures between 0—300 mm Hg, and with graduated water filled tubes for differential pressures between 0—20 cm H_2O — ranges within which a linear relationship was obtained between pressure and galvanometer deflection The calibration was checked after each experiment and the sensitivity was found to be highly stable

Dynamic calibration of the system — from catheter to ink writer — was done by applying a sinusoid pressure to each of the catheter openings separately and also to both of them simultaneously The pressure generator was of Elmqvist s design, modified for this particular purpose. The pressure in the generator chamber was monitored by a Swema transducer via a short metal cannula. The response of the monitoring gauge was accurate up to approximately 40 c/sec The pressure signals recorded by this system as well as the signals from the catheter manometer system were fed to the X and Y plates of a Tektronix Type 532 oscilloscope to obtain Lissajou figures from which the phase angle could be calculated The frequency response is shown in Fig 8 In this diagram the ratio of amplitude response to amplitude of the lowest driving frequency and the phase lag are plotted as functions of the ratio of driving frequency to resonant frequency measured at amplitude peak When the same pressure was applied to both catheter openings to test the dynamic *unbalance* a zero response was obtained up to 15 c/sec The above measurements were made at approximately 18° C room temperature Similar measurements with the catheter immersed in a water bath at about 37° C revealed no appreciable difference in performance

The integrated pressure signal and the pressure were recorded simultaneously to check the performance of the integrating circuits at a fixed RC constant of 0 02 sec. For this setting integration was found to be accurate up to about 10 c/sec.

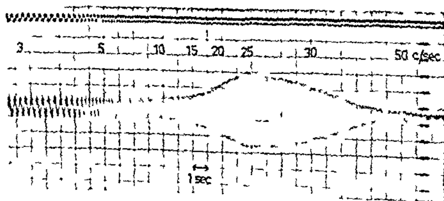


Fig 9 Amplitude response recorded at slow paper speed when the catheter manometer system is driven by an oscillatory differential pressure Peaking at about 25 c/sec

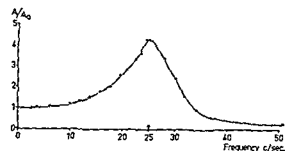


Fig 10 Amplitude response as a function of frequency for the differential pressure performance

The author had the opportunity of checking many of the calibration results by similar measurements done in collaboration with Shillingford and Gabe at the Hammersmith *Postgraduate Medical School, London The pressure generator designed by* Ball and Gabe was used for these measurements An advantage of the British pressure generator is that a sinusoid differential pressure can be applied to the catheter manometer system This test is particularly interesting in that it demonstrates the effect of coupling within the closed system The amplitude responses at these calibrations are presented in Figs 9 and 10 For the phase lag no appreciable deviation from the results given in diagram 8 was detected

By the sudden withdrawal of a syringe plunger a transient pressure impulse was imposed on each catheter opening The damping calculated from the measured logarithmic decrement, was found to be 0 1—0 2 of critical damping

Comment

The calibration of the catheter manometer system under different experimental conditions shows that the amplitude response for frequencies up to 10—12 c/sec is within 10 % of the low frequency response (about 1 c/sec) Within the same range of frequencies the phase lag is less than 5 degrees These results apply to the unilateral performance but are essentially the same for differential performance When the same pressure is applied to both catheter openings a zero response is obtained up to about 15 c/sec

In assessing the adequacy of the system s performance the frequency components of the ascending aortic pulse wave and the differential pressure must be considered As shown first by Porjé (1946) and subsequently by other workers e g Wood (1956) and Randall (1958), the first two harmonics dominate the Fourier series representing the arterial pulse wave On analysis of the ascending aortic pulse pressure in two cases (Nos 28 and 29) with respect to five harmonics, the first two harmonics are found to dominate the spectrum and the amplitude of the fifth harmonic is only a small fraction of the fundamental frequency Similar analysis of the differential pressure indicates that harmonics higher than the fifth might not be inconsiderable For frequencies above 10—12 c/sec, amplitude and phase distortion will increase

rapidly The low damping of the system will make it "ring" at resonance frequency Superimposed resonance oscillations will disturb the differential pressure record to a varying degree, as will be shown For reasons implicit in the theory, the amplitudes of the flow terms are inversely proportional to the order of the corresponding harmonics in the differential pressure wave form (See pages 52 and 53.) Calculation of flow from the differential pressure without correction for the distortion of the higher frequencies will not, therefore, entail too serious an error, and for calculation of stroke volume the error should be very small In short, the performance of the system is adequate for calculation of instantaneous flow and stroke volume from the differential pressure record

CHAPTER 3

METHODS

Preparation of the Apparatus

To ensure adequate stability the apparatus is switched on two hours before the examination The manometer and one pair of stopcocks are carefully cleaned with a detergent (benzalkonium chloride 50 % 2 g, aceton 80 g, spir conc 96 % 10 g, aqua dest ad 400 g) and the manometer with connected stopcocks is then filled with freshly boiled distilled water Another pair of stopcocks is sterilized by boiling and then connected to the catheter, which has been cold sterilized for 24 hours in a liquid composed of Septin 10 g, sodium phosph tribas 50 g, and aqua dest ad 5000 g The catheter with three way stopcocks is immersed in a saline filled container and the connecting tubes from two bottles containing heparinized saline are each fitted to one branch of a stopcock The catheter is perfused until air bubbles cease to escape from the catheter openings The stopcocks are then closed and the catheter is ready for insertion into the artery The exclusion of air bubbles from the whole catheter-manometer system is of fundamental practical importance in this procedure since even small air bubbles can substantially reduce the frequency response Painstaking precau tions are therefore essential

Introduction of the Catheter

In catheterization of the ascending aorta the catheter is inserted into a peripheral artery For the insertion of a closed tip catheter for intra arterial injection of contrast medium the author has, for several years used a cannula described by Gidlund (1956) It seemed appropriate therefore to use this instrument for insertion of the double lumen catheter as well It was thought that a modification of the cannula would render it more suitable for this purpose Figure 11 shows the modified instrument, which is, in principle, the Gidlund cannula cut into two halves that can be separated to leave the catheter free With this device the catheter can be introduced into the femoral artery without harm to the patient It is inadvisable, however to use this rather heavy cannula for puncturing the brachial artery since it might cause injury to the vessel Catheterization from the right arm is advantageous in that a shorter catheter can be used and positioning of the catheter in the ascending aorta can be readily accomplished The present catheter however, is of such diameter that this procedure

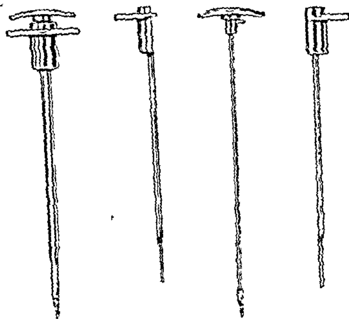


Fig 11 Cannula for insertion of double-lumen catheter

necessitates surgical exposure of e g the radial artery *ad modum* Radner (1948) It was possible therefore to utilize this route only in those few cases where the artery was exposed for other purposes At transfemoral catheterization of the ascending aorta the end of the catheter should be pre bent to fit the curvature of the aortic arch (Ödman and Philipsson 1958) — a measure which greatly facilitates positioning

One of the femoral arteries is punctured in the groin under local anesthesia The inner cutting mandrel of the cannula is withdrawn and the catheter is inserted through the outer cannula which is then taken apart to leave the catheter free for further manipulations Although bleeding at the puncture site can usually be arrested by slight manual compression mechanical compression is sometimes useful, especially when the catheter has to be rotated repeatedly For mechanical compression an instrument designed by Gidlund has proved serviceable During these manipulations the catheter is continuously perfused It is advanced to the ascending aorta under fluoroscopic guidance — a procedure which is greatly facilitated by the use of an image intensifier and a television system. The catheter tip should be advanced gently towards the aortic valves and then withdrawn a short distance This procedure gives some idea of the length of the ascending aorta and the position of the aortic orifice The

stopcocks[1] at the end of the catheter are then screwed to those on the manometer All connections within the system should now be perfused in such a way that no liquid enters the catheter by way of the manometer, since this part of the system cannot be considered sterile Figure 12 shows the arrangement

Recording of the Differential Pressure

It is convenient as a first step, to record the aortic pressure at low gain from both measuring points separately and then the differential pressure When both pressures are transmitted to the manometer a straight line should be registered by the recording device — the ink writer or the oscilloscope This line should coincide with the pre set zero line The amplification is gradually increased until a suitable amplitude of the differential pressure is reached When as frequently happens high frequency oscillations are superimposed on the differential pressure curve the fluoroscopic or television screen will show the catheter moving rapidly at each heart stroke By small translations and rotations this motion can usually be reduced with a commensurate decrease of the high frequency oscillations By inspection of the pressure curves it is generally possible to ascertain whether or not the frequency response of the system has diminished Asymmetry within the system will also cause

[1] Stopcocks EMT 473 manufactured by Elema Schonander AB Stockholm Solna Sweden

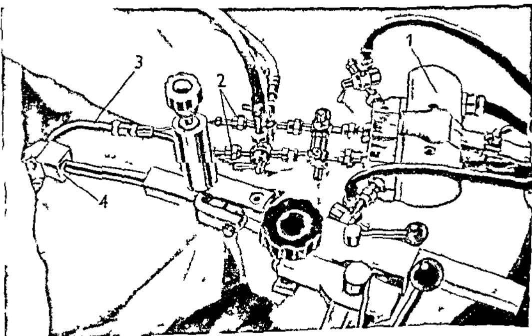


Fig 12 Photograph of pressure transducer (1) stopcocks (2) and double-lumen catheter (3) which is introduced into the right femoral artery in the groin Instrument for mechanical compression (4)

the differential pressure at low gain to deviate from a straight line If any doubt should remain concerning the frequency response, pressure signals from the catheter should be switched to the opposite side of the manometer Since this manipulation will alter only the sign of the differential pressure when both sides have identical frequency response it constitutes a highly sensitive test of symmetry It is, of course, conceivable that the frequency response could have dropped equally on both sides of the system without affecting the shape of the pressure curves or altering the symmetry To detect a slight symmetrical drop in frequency response it would be necessary to measure the response immediately after each examination With the present equipment this would be a rather tedious procedure and, for practical reasons, was not attempted here Measurement of the resonance frequency however, is in general sufficient in this situation Such a test is easily performed by applying an impulse pressure to the catheter openings or by tapping the catheter and recording the resultant resonant oscillations By these measures it is possible to determine whether the frequency properties of the recording system differ appreciably from those obtained *in vitro* As in all intravascular pressure measurements, records should be taken only over brief periods of time separated by intervals of slow perfusion

A convention adopted here is that the pressure from a point situated in the proximal part of the ascending aorta is recorded with an upward deflection, and that from a distal point with a downward deflection

Determination of the Density of Blood

The density of blood can either be measured directly (e g Phillips *et al*, 1950) or taken from tables relating hematocrit and total protein to density (e g van Slyke *et al*, 1950) From such tables it is found that normal values for density vary between 1 050—1 056 g/cm³ in females and 1 055—1 062 g/cm³ in males Since the subjects studied here had no abnormalities as respects blood composition, a standard value of 1 06 g/cm³ was used for the calculations The error introduced by ignoring individual variations should be of the order of magnitude of 1 %

Determination of the Distance between the Catheter Side Holes and of the Cross Sectional Area of the Aorta

To quantitate the wave form of instantaneous blood acceleration and velocity, the distance between the pressure measuring points along the axis of the aorta must be known This distance, which we may call the effective distance and denote by L, will generally differ from the distance between the catheter openings measured along the catheter For exact determination of the effective distance the geometry of the ascending aorta as well as the position of the catheter must be known In the absence of such information a reasonable approximation of the effective distance can be obtained from measurements on two radiograms exposed at right angles to each other

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1 Stopcocks EMT 473 manufactured by Elema Schonander AB Stockholm Solna Sweden

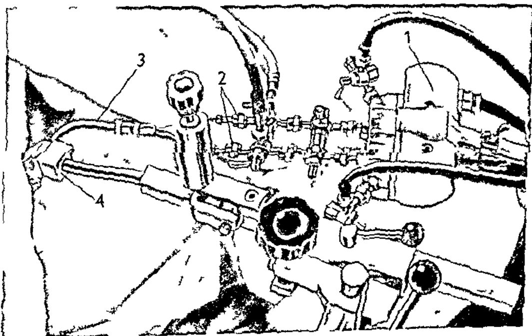


Fig 12 Photograph of pressure transducer (1) stopcocks (2) and double-lumen catheter (3) which is introduced into the right femoral artery in the groin Instrument for mechanical compression (4)

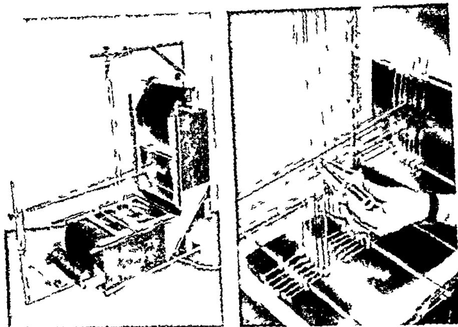


Fig 14 Equipment for determinat on of cross sectional area of the aorta and effective length between the catheter s de holes Photograph on the right is detail showing measurement of as end ng aortic d ameter by means of a cone graded into diameters See page 28

The calculation of instantaneous ascending aortic blood flow and cardiac output requires according to theory determination of the cross sectional area of the aorta. Such measurements are difficult to perform in human subjects and at the present time could not be made without the aid of radiology For an accurate determination of effective distance and cross sectional area rapid serial bi plane angiography is the most appropriate technique This also permits study of the cross sectional area *(Q(x t))* as a function of time thus making it possible to calculate the correctional term in eq (5) for the elastic deformation of the aorta Detailed information on the anatomy of the ascending aorta and the aortic valve is of importance since full application of the theory presupposes a competent aortic valve and no abnormalities in the ascending aorta For application of the theory it is also essential to know the position of the catheter in relation to various aortic landmarks such as the valve the ostia of the coronary arteries and the branching of the innominate artery

For determination of the cross sectional area of the aorta by angiography a bi plane film changer designed by Gidlund (1956) was employed By direct measurement on radiograms exposed in two perpendicular planes the cross sectional area can be calculated if it is assumed that the cross section is at each level circular Approximate

A correction for the geometrical magnification on the films can be made by measuring the distances involved The length of the gold inserts in the catheter can also be used for this purpose With the patient supine the axis of the ascending aorta is in an almost horizontal plane and is only slightly curved in the lateral projection The length, l_1, of the horizontal projection of the distance between the side holes, measured on a radiogram exposed in the lateral projection, would be equal to the effective distance, L, if the aorta were a *straight cylindrical tube parallel to the film* plane and the holes were situated along the tube axis Since the ascending aorta usually is slightly curved even in the frontal projection, and the side holes, as a rule, are not along the axis, distance l_1 is likely to be shorter than the effective distance (fig 13)

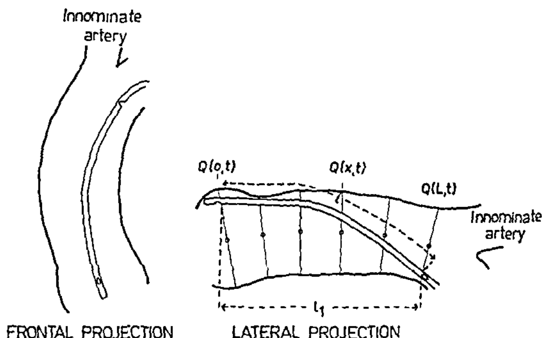


Fig 13 Contours of the double-lumen catheter and the ascending aorta redrawn from radiograms obtained in case 31 Length l_1 is the horizontal projection of the distance between the catheter side holes, and length l the distance measured along the catheter Q denotes the cross sectional area at different levels of the aorta

The distance l between the catheter side holes *measured along the catheter* would be equal to the effective distance if the ascending aorta were a straight cylindrical tube and the catheter lay along the tube axis As the catheter is pre bent and usually slightly curved in the ascending aorta, distance l is likely to be longer than the effective distance, the value of which should fall somewhere between the two values l and l_1 The mean value of l and l_1 is therefore used for quantitation of velocity and acceleration in cases where the exact geometry of the ascending aorta is unknown

CHAPTER 4

CLINICAL MATERIAL

The conditions requisite for the measurement of instantaneous blood flow and other hemodynamic data in the ascending aorta have been discussed in the preceding sections The problems involved in the application of the differential pressure method to human beings were studied in a series of 31 cases In four cases it was not possible to position the catheter in the ascending aorta and in eight cases there were signs of aortic valvular disease The differential pressure pattern found in aortic valvular stenosis has been described by Porje and Rudewald (1959) The aortic valves of the remaining 19 cases were normal as judged from careful clinical examination Seven of these patients were studied with right heart catheterization angiography and differential pressure measurement by Karnell Porjé, Rudewald and Stenson, and some of the results were presented at a meeting of the Swedish Society of Cardiology in 1961 The remaining 12 cases were studied by the author with differential pressure measurement only The effect of a nitrate compound was investigated in seven of these cases and preliminary data hereon were reported by Porje and Rudewald in 1961 The group of 12 subjects comprised two normals and 10 patients admitted to the Geriatric Clinic of Sodersjukhuset for investigation of various diseases The differential pressure measurements were carried out at the Roentgen Department of St Erik s Hospital There were three cases of pseudoxanthoma elasticum, three cases of hypertension one case of peripheral vascular disease one case of total heart block, one case of mitral stenosis and one case of orthostatic hypotension The arterial pressure was recorded at various levels of the aorta The differential pressure pattern found in the ascending aorta will be described in detail in the next chapter In these cases it was possible to quantitate the wave forms of acceleration and blood velocity by the determination of blood density and effective length The instantaneous blood velocity $U(t)$ was calculated by graphic integration of the differential pressure (formula (6)) and the instantaneous blood acceleration was determined from formula (8) The quotient of differential pressure and effective distance — the pressure gradient — was determined from the calibrated differential pressure curve The characteristics of blood velocity and acceleration curves were related to the electrocardiographic events via synchronous records

corrections for the geometrical magnification can be made by measuring the focus film distance and the distance from any given point on the aorta to the film to obtain the factor by which the measured diameters are to be multiplied A detailed analytical treatment involves rather complex expressions and the geometry of the problem will differ from case to case It was therefore thought feasible to construct a simple simulator for the direct measurement of aortic diameters This equipment shown in Fig 14 consists of two mutually perpendicular light boxes representing the two film planes The film is fed over these boxes precisely as in angiographic examination The focal spots which are given the same dimensions they have in the roentgen tube are positioned at the distance used for angiography The central ray from the focus can be identified on the film by an indicator or from the outlines of a diaphgram From the periphery of the focal spot nylon threads are drawn to the contour of the aorta on the film thus simulating roentgen rays Two pairs of threads one pair from each focal spot combine to give a quadrangular figure in space in which a circle could be inscribed By combining several pairs of threads a three dimensional figure is obtained from which the diameters of the ascending aorta can be measured directly For this purpose a cone graded into diameters is useful The location of the catheter within this figure can be indicated for determination of the effective distance

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either with pressure or with differential pressure On radiograms referable to different phases of the cardiac cycle the variations of the aortic dimensions could be studied The cross sectional area of the ascending aorta used for the calculations was determined from the mean of 6—8 values from different parts of the vessel between the pressure measuring points It was also possible to plot the mean cross sectional area of the aorta against time, and so study the effect of the elastic deformation of the vessel A full graphical calculation of the correctional term in eq (5) was made in case 31

The differential pressure was electrically integrated in these seven cases to give the wave form of instantaneous blood flow synchronously with the differential pressure The resulting flow curve was also checked by graphical integration The wave form of blood flow was quantitated by determining the density of blood, the effective distance and the mean cross sectional area of the ascending aorta in accordance with the principles discussed above The stroke volume was calculated from formula (7) with $t=0$ at the end of diastole and $t=t_1$ at that point on the time axis where the flow curve had returned to zero level The mean flow calculated from a series of heart beats during the Fick period was used in determining the cardiac output For timing of certain hemodynamic events the peaking of the R wave on the ECG was used as reference Since the exact time realtionship between electrical and mechanical events was not known, it seemed feasible to use the most clearly defined electrical event as a reference Potential and kinetic energy delivered by the heart to the aorta was calculated from formulae (9) and (10) in cases 28, 29, 32 and 33 For comparison the potential energy was also calculated in the conventional manner from mean pressure and mean flow

The calculation of instantaneous aortic blood flow by the differential pressure method involves, first, a series of assumptions which are defensible, within certain limits, and, secondly, measurements which will inevitably entail experimental errors It would, therefore, be of great interest to compare the results of this method with those obtained by another technique Unfortunately, other techniques do not exist for the measurement of instantaneous aortic blood flow in intact man The mean blood flow in the ascending aorta as calculated from the differential pressure record can however, be compared to cardiac output as determined by conventional methods In the present work values for cardiac output as determined by the Fick method have been used for comparison Data sufficient for the comparison have been obtained in the seven cases referred to above These patients were admitted to the Heart Clinic, Soder sjukhuset, for cardiac investigation, and right heart catheterization and angiography were carried out for diagnostic purposes The examinations were done at Roentgen Department I, Sodersjukhuset For right heart catheterization and the angiographic procedure the routine developed by Jonsson, Karnell and co workers was followed

One hour before the examination the patients were given 0 1 g pentobarbital and 0 5 mg morphine scopolamine subcutaneously (Arvidsson, 1958) The right heart and the pulmonary artery were catheterized from a cubital vein After pressure measurements had been taken from various positions, the catheter was positioned in the pulmonary artery The double lumen catheter was introduced percutaneously from one of the femoral arteries — usually the right — in accordance with the procedure previously described In two patients (cases 28 and 30) the radial artery, which had been exposed for the purpose of thoracic aortography and left ventricular angiography respectively, was used for insertion of the double lumen catheter An arterial needle was introduced into one of the brachial arteries for pressure measurements and blood sampling

After a resting period of about 10 minutes the patient was connected to a spiro meter system (Spirograph IV, Elema Schonander AB) and allowed to breath pure oxygen for about five minutes During the next 10 minutes the oxygen consumption was measured and midway through this period blood samples were drawn from the pulmonary artery and from a peripheral artery simultaneously The differential pres sure was recorded repeatedly at the beginning, the middle and the end of the 10 minute interval

These measurements were followed by the angiographic procedure under general anaesthesia (Jonsson, Broden and Karnell 1949, Arvidsson, 1958) Selective angio cardiography was performed in five cases and the left heart and the the aorta were opacified in four cases In two cases the aorta was visualized by injection of contrast medium into the aorta or the left ventricle During opacification of the left heart and the aorta six exposures were made in the frontal and lateral projections The chronological relation of the exposures to the heart cycle was determined by reference to a simulaneously recorded electrocardigram The records were taken synchronously,

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After a resting period of about 10 minutes the patient was connected to a spiro meter system (Spirograph IV, Elema Schonander AB) and allowed to breath pure oxygen for about five minutes During the next 10 minutes the oxygen consumption was measured and midway through this period blood samples were drawn from the pulmonary artery and from a peripheral artery simultaneously The differential pres sure was recorded repeatedly at the beginning the middle and the end of the 10 minute interval

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CHAPTER 5

RESULTS

The results will be presented under three headings First, the differential pressure pattern found in the ascending aorta will be described in qualitative terms Secondly, the instantaneous blood velocity, acceleration and other hemodynamic data will be *set forth in detail Thirdly, data will be given for the seven cases in which the* differential pressure measurements were taken in conjunction with angiography and right heart catheterization

Differential Pressure Pattern in the Ascending Aorta

During the series of aortic catheterizations the differential pressure pattern was *observed to show certain characteristics which appeared to be correlated to the posi*tion of the catheter in the ascending aorta A description of the differential pressure wave form as the catheter is withdrawn from the left ventricle to the proximal aorta *may serve to illustrate these phenomena In the following instance* the distance between the two pressure measuring points was approximately 8 mm

(i) Position I Fig 15 Here the proximal opening of the catheter was situated within the left ventricle and the distal opening was in the aorta At the end of diastole the aortic pressure exceeded the intraventricular pressure by approximately 70 cm H_2O During systole the pressure difference between the two measuring points became very small — too small indeed to be accurately measured with the scale used On closure of the aortic valve the pressure difference returned to negative

(ii) Position II Fig 15 The catheter was withdrawn until its forward end was at the level of the aortic valve From this position the ventricle could be re entered without difficulty and it seems likely, therefore that the catheter was held during diastole by the aortic valve cusps — It will be seen that at the end of diastole there is no detectable pressure difference between the two holes of the catheter In early systole there is a positive pressure gradient resulting from acceleration of blood in the aorta, followed by a prolonged negative gradient In early diastole there is still a slight negative pressure gradient

(iii) Position III, Fig 15 With the proximal hole of the catheter about 1 cm above the valve the wave form of the differential pressure diverges somewhat from that

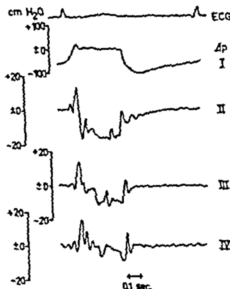


Fig 15 Redrawn differential pressure curves from various positions in the ascending aorta referred to in the text The sensitivity is increased by a factor of about 8 in the three lower records

in the other positions In this position the catheter was seen to move back and forth synchronously with each heart beat — The pressure gradient is zero in late diastole, becoming positive in early systole and then negative as systole proceeds It should be observed that the negative part of the curve is less pronounced than in position II During diastole the curve is on a uniform level and there is no significant pressure difference between the two catheter holes This is the pattern normally observed when the tip of the catheter is contiguous to the aortic valve

(iv) Position IV Fig 15 A further very slight withdrawal of the catheter *affects* principally the negative phase of the differential pressure curve as shown in the figure The movements of the catheter are now virtually eliminated There is again a positive pressure gradient in early systole but the negative phase is shorter than that shown in positions II and III At the end of systole there are sharp deflections, probably resulting from closure of the aortic valve

Comment — It is interesting to compare these observations with those referable to the shape of the differential pressure wave form in aortic valvular stenosis (Porje, and Rudewald 1959) There the negative phase of the differential pressure curve from a point close to the valve entirely dominated the curve With increasing distance from the valve the differential pressure pattern showed alteration similar to that described above but it was *necessary to* withdraw the catheter a much greater di

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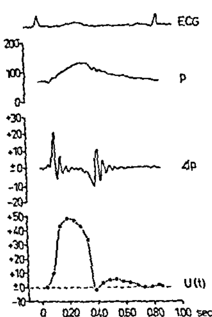


Fig 17 Case 20 Woman, 49 with pseudoxanthoma elasticum Redrawn curves for pressure in mm Hg and differential pressure in cm H_2O Lower curve shows instantaneous blood velocity in cm/sec

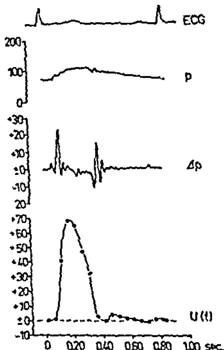


Fig 18 Case 21 Healthy man, 39 Redrawn curves for pressure in mm Hg and differential pressure in cm H_2O Lower curve shows instantaneous blood velocity in cm/sec

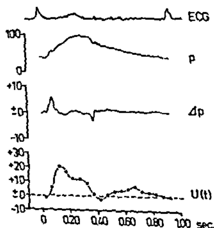


Fig 19 Case 26 Woman, 64 with orthostatic hypotension Redrawn curves for pressure in mm Hg and differential pressure in cm H_2O Lower curve shows instantaneous blood velocity in cm/sec

stance from the valve in order to reduce the negative phase This effect was ascribed to the particular pressure distribution of the expanding jet caused by the narrowing of the aortic orifice It was possible to reproduce this effect in a model circulatory system The similarity of these phenomena to those observed in cases with normal valves suggest that a region close to the normal aortic valve might have the character of an expanding ' jet

Instantaneous Aortic Blood Velocity and Acceleration

The results presented here are collected from 12 examinations made on six male and six female subjects ranging in age from 27 to 64 years The measurements were made without pre medication and with the patient supine The double lumen catheter was inserted percutaneously into one of the femoral arteries The catheter tip was then advanced to the aortic valve and adjusted to a position close to the valve In the final catheter position two perpendicular radiograms were exposed and by measuring the distances involved the factor for correction of the geometrical magnification was determined in the lateral projection No complications arose during or after these examinations

Curves for pressure, differential pressure and velocity from some of the cases are shown in Figs 16 to 20 They were chosen to illustrate various velocity curves

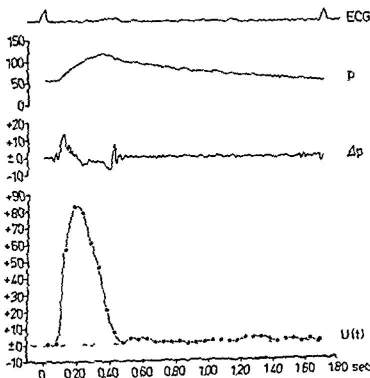


Fig 16 Case 10 Man 59 with total heart block Redrawn curves for pressure in mm Hg and differential pressure in cm H O Lower curve shows instantaneous blood velocity in cm sec

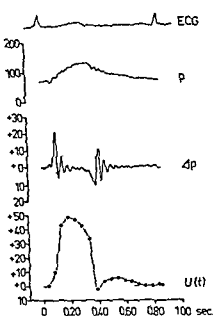


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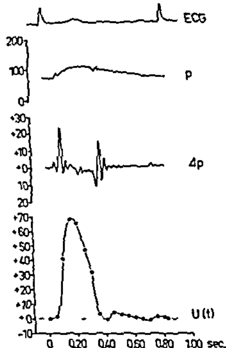


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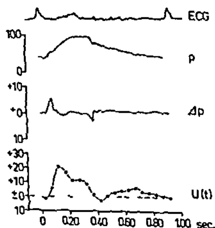


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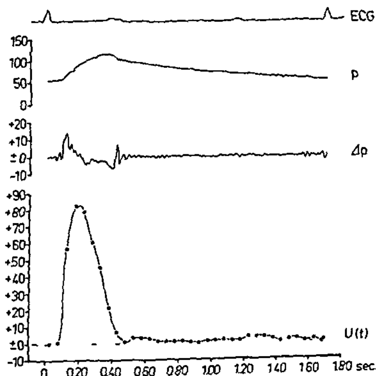


Fig 16 Case 10 Man 59 with total heart block Redrawn curves for pressure in mm Hg and differential pressure in cm H O Lower curve shows instantaneous blood velocity in cm/sec

ranges between 0 26 and 0 35 sec The maximum pressure lags behind the maximum velocity by 0 10 to 0 18 sec

Series of data from the 12 cases are collected in Table I It will be seen that the maximum differential pressure (Δp_{max}) varies between 6 and 22 cm H_2O and the maximum pressure gradient between 0 7 and 2 9 cm H_2O per cm The maximum acceleration (a_{max}) expressed as a fraction of the acceleration due to gravity is found to be 0 7 · g in a 64 year old woman with orthostatic hypotension and 2 7 · g in a 39 year old healthy man The quotient of U_{max} and the time interval $t_{R-U_{max}}$, corresponding to the mean acceleration during that time interval, is denoted by a_{mean} and is also expressed as a fraction of g The relationship between mean acceleration and age is shown in Fig 21, which also gives the values for the other group of seven patients The mean acceleration tends to become lower with increasing age It is interesting to note that the values for the male subjects are slightly higher than those for the females

Instantaneous Aortic Blood Flow and Cardiac Output

In this section the results from the seven cases studied by means of right heart catheterization angiography and differential pressure measurement, as suggested by Porje and Rudewald (1957) will be presented These examinations were conducted on the basis of team work between members of the staff of the Geriatric Clinic, the Heart Clinic and Roentgen Department I, Södersjukhuset A comprehensive re-

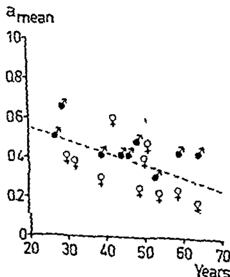


Fig 21 Mean acceleration expressed as a fraction of the acceleration due to gravity, is plotted against age Broken line represents the regression line

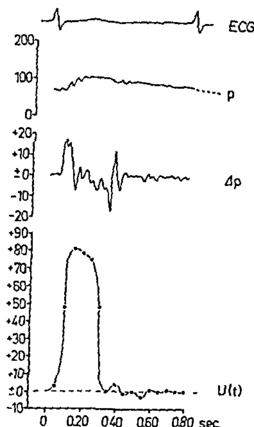


Fig 20 Case 27 Healthy man, 27 Redrawn curves for pressure in mm Hg and differential pressure in cm H_2O Lower curve shows instantaneous blood velocity in cm/sec

from patients of different ages and with different diagnoses The records of pressure and differential pressure were not taken synchronously, and the curves demonstrated are redrawn from heart beat series separated by short intervals The duration of the heart beat was almost constant The velocity curve, starting from a zero value, rises rapidly to a maximum value and then falls off more slowly to a zero value The phase of positive velocities is followed by a short negative deflection, after which the velocities are close to zero during the remaining part of the period This behaviour was observed in eight cases In one case the velocity curve was positive during most of the period, in two cases the phase of positive velocities was followed by a prolonged phase of slighty negative velocities, and in one case this phase was very pronounced These deviations from the rule were considered artefacts and will be discussed later on (page 57) The time interval from the peaking of the R wave on the ECG to the instant of maximum velocity (U_{max}) is denoted by $t_{R-U_{max}}$, and varies from 0 11 to 0 21 sec The time interval from the peaking of the R wave to the instant of maximum pressure, denoted by t_{R-p} .

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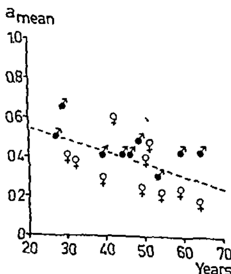


Fig 21 Mean acceleration, expressed as a fraction of the acceleration due to gravity is plotted against age Broken line represents the regression line

Table I

Hemodynamic data collected from 12 cases

Case No	Age	Sex	Diagnosis	Δp_{max} cm H_2O	L cm	$\frac{\Delta p_{max}}{L}$	a_{max} g	U_{max} cm/sec	$t_{R-U_{max}}$ sec	$t_{R-p_{max}}$ sec	a_{mean} g
10	59	♂	Total heart block	15	6 7	2 2	2 1	82	0 20	0 35	0 42
16	64	♂	Peripheral vascular disease	11	7 5	1 5	1 1	54	0 13	0 26	0 42
17	59	♀	Hyperten sion	12	7 5	1 7	1 6	55	0 23	0 33	0 24
19	30	♀	Mitral stenosis	19	7 9	2 4	2 3	53	0 13	0 31	0 41
20	49	♀	Pseudo xanthoma elasticum	21	8 7	2 4	2 3	49	0 20	0 33	0 25
21	39	♂	Normal	22	7 7	2 9	2 7	68	0 17	0 31	0 41
22	46	♂	Hyperten sion	17	8 7	2 0	1 9	44	0 11	0 28	0 41
23	50	♀	Pseudo xanthoma elasticum	14	7 7	1 8	1 7	74	0 19	0 31	0 40
24	53	♂	Hyperten sion	16	9 0	1 8	1 7	53	0 18	0 29	0 30
25	39	♀	Pseudo xanthoma elasticum	14	7 8	1 8	1 7	64	0 21	0 32	0 30
26	64	♀	Orthosta tic hypo tension	6	7 3	0 7	0 7	21	0 12	0 30	0 18
27	27	♂	Normal	17	8 4	2 0	1 9	81	0 16	0 31	0 50

port on the clinical aspects of these examinations is being prepared by Karnell, Porje, Rudewald and Sténson

Synchronous electrocardiographic, differential pressure and integrated differential pressure records are given for each subject In all cases except No 32 the deflection caused by the differential pressure signal was recorded in conformity with the convention adopted earlier (see page 25) A symmetry test was performed in case 32 with the catheter endings reversed and the differential pressure signal fed through a filtering network with an upper cut off frequency of 20 c/sec prior to entering the ink writer Since the deflection of the differential pressure signal, after being reversed by the first procedure, was restored by the filtering network, the curve has the conventional orientation The filtering network, it was found, introduced distortion of the differential pressure signal and the resulting curve must therefore, in this instance, be regarded as a qualitative record In all cases, however, the differential pressure signal was fed unfiltered to the integrator Thus the wave form of flow in case 32 was not distorted by the filtering network and the deflection was negative because of reversal of the catheter endings The wave form of flow was calibrated in accordance with the aforementioned principles In case 34 the aorta was not opacified and, for calibration of the flow curve, it was assumed that the quotient of cross sectional area and effective distance was equal to one

Values for cardiac output as determined by the Fick method and the differential pressure methods are plotted for comparison in Fig 22 It will be seen that in four cases

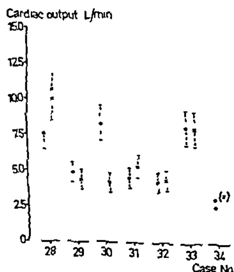


Fig 22 Values for cardiac output determined by the Fick method (heavy dots) and the differential pressure method (crosses) The experimental error (±15 %) is indicated by broken lines

Table I

Hemodynamic data collected from 12 cases

Case No	Age	Sex	Diagnosis	Δp_{max} cm H_2O	L cm	$\frac{\Delta p_{max}}{L}$	a_{max} g	U_{max} cm/sec	$t_{R-U_{max}}$ sec	$t_{R-p_{max}}$ sec	a_{mean} g
10	59	♂	Total heart block	15	6 7	2 2	2 1	82	0 20	0 35	0 42
16	64	♂	Peripheral vascular disease	11	7 5	1 5	1 1	54	0 13	0 26	0 42
17	59	♀	Hyperten sion	12	7 5	1 7	1 6	55	0 23	0 33	0 24
19	30	♀	Mitral stenosis	19	7 9	2 4	2 3	53	0 13	0 31	0 41
20	49	♀	Pseudo xanthoma elasticum	21	8 7	2 4	2 3	49	0 20	0 33	0 25
21	39	♂	Normal	22	7 7	2 9	2 7	68	0 17	0 31	0 41
22	46	♂	Hyperten sion	17	8 7	2 0	1 9	44	0 11	0 28	0 41
23	50	♀	Pseudo xanthoma elasticum	14	7 7	1 8	1 7	74	0 19	0 31	0 40
24	53	♂	Hyperten sion	16	9 0	1 8	1 7	53	0 18	0 29	0 30
25	39	♀	Pseudo xanthoma elasticum	14	7 8	1 8	1 7	64	0 21	0 32	0 30
26	64	♀	Orthosta tic hypo tension	6	7 3	0 7	0 7	21	0 12	0 30	0 18
27	27	♂	Normal	17	8 4	2 0	1 9	81	0 16	0 31	0 50

Case 28 Woman 42, with aortic coarctation

Roentgen data Thoracic aortography demonstrated a characteristic isthmus stenosis with collateral vessels The cusps of the aortic valve appeared to be slightly thickened, but their movements seemed normal There were no signs of valvular stenosis or incompetence The ascending aorta was curved and slightly dilated in its middle part The distal opening of the catheter was just above the origin of the innominate artery

$Q_m = 7.1$ cm² $L = 7.3$ cm Q_m varied by less than 5 per cent of the end diastolic value

Fick data Oxygen consumption 198 cm³ min
A V O_2 diff 26 ml L
Cardiac output 7 6 L/min
Heart rate 85 beats min
Stroke volume 90 cm³

Differential pressure and flow

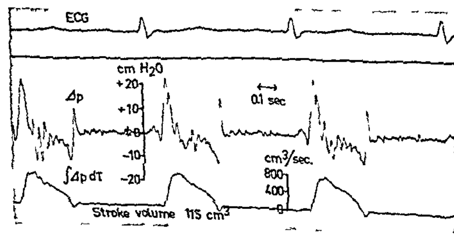


Fig 23 Calibrated curves for differential pressure and instantaneous blood flow

there is good agreement between the two methods In case 28 the value determined by the differential pressure technique was higher, and in case 30 lower than that determined by the Fick method The experimental error involved in the differential pressure method is estimated to be of the order of ± 15 per cent, and is indicated by broken lines in the diagram For purposes of comparison the Fick values were assumed to have the same relative error

Data measured from differential pressure and flow curves are collected in Table II The maximum values of the pressure gradient and acceleration are found to be 3 1 cm H_2O per cm and 2 9 *g* respectively in a 29 year old man with mitral stenosis In this case (No 33) the mean acceleration (see page 52), as measured is 0 67 · *g* — the highest value found in the series The time interval from peaking of the R wave to the instant of maximum instantaneous flow, $(i_m)_{max}$, is denoted by $t_{R-(i_m)_{max}}$ In case 34 that interval is 0 21 sec, and the flow reaches its maximum 0 06 sec before the pressure is at maximum

Table II

Hemodynamic data collected from 7 cases

Case No	Age	Sex	Diagnosis	p_{max} cm H_2O	L cm	$\frac{p_{max}}{L}$	a_{max} g	$(i_m)_{max}$ cm³/sec	$t_{R-(i_m)_{max}}$ sec	$t_{R-p_{max}}$ sec	a_{mean} g
28	42	♀	Aortic coarctation	22	7 3	3 0	2 8	780	0 17	0 28	0 65
29	51	♀	Mitral stenosis	19	7 1	2 7	2 6	390	0 11	0 27	0 49
30	44	♂	Pericardial tumor	18	7 1	2 5	2 4	480	0 16	0 28	0 41
31	48	♂	Mitral stenosis	17	8 5	2 0	1 9	480	0 17	0 27	0 49
32	32	♀	Mitral stenosis	15	7 0	2 1	2 0	350	0 15	0 28	0 10
33	29	♂	Mitral stenosis	23	7 5	3 1	2 9	650	0 15	0 22	0 67
34	54	♀	Mitral stenosis	12	7 0	1 7	1 6	(310)	0 21	0 27	0 21

Case 29 Woman 51, with mitral stenosis

Roentgen data Angiocardiography demonstrated typical mitral stenosis The aortic valve was normal

$Q_m = 7\ 4$ cm² $L = 7\ 0$ cm Q_m varied by less than 4 per cent of the end diastolic value

Fick data Oxygen consumption 177 cm³/min
A V O_2 diff 36 ml/L
Cardiac output 4 9 L/min
Heart rate 62 beats min
Stroke volume 80 cm³

Differential pressure and flow

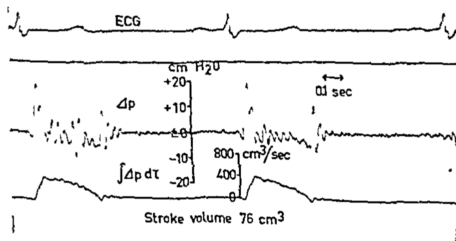


Fig 25 Calibrated curves for differential pressure and instantaneous blood flow

The mean flow was stable during the Fick period The cardiac output was estimated at 4 4 L/min

The stroke volume of consecutive beats was estimated in the early, mid and late phases of the Fick period (fig 24) It will be observed that the stroke volume and the heart rate changed during this period The mean flow tended to increase and the cardiac output increased from 10 0 to 10 7 L/min

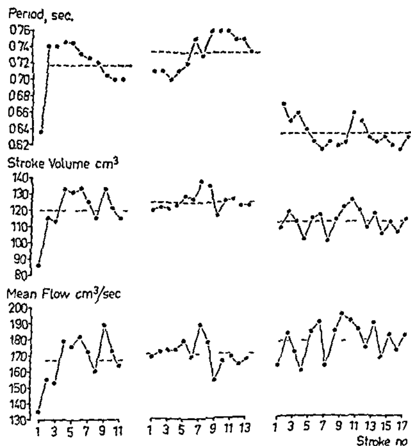


Fig 24 Period (heart cycle duration) stroke volume and mean flow of 11 13 and 17 consecutive hearts from early mid and late phases of the Fick period Broken lines indicate mean values

Case 31 Man, 48 with mitral stenosis Digital valvulotomy had been performed 8 years earlier

Roentgen data Angiocardiography demonstrated typical mitral stenosis The aortic valve was normal

$Q_m = 5\ 9$ cm² $L = 08\ 5$ cm Q_m varied by less than 7 per cent of the end diastolic value

Fick data Oxygen consumption 196 cm³/min
A V O_2 diff 44 ml/L
Cardiac output 4 5 L/min
Heart rate 70 beats min
Stroke volume 64 cm³

Differential pressure and flow

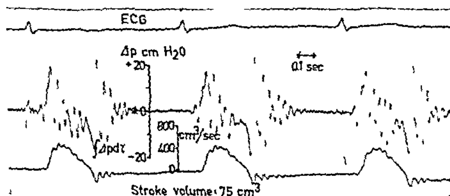


Fig 77 Calibrated curves for differential pressure and instantaneous blood flow

The flow curve drops slightly below the end diastolic level at the end of systole, as confirmed by graphical integration The mean flow was stable during the Fick period The calculated cardiac output is 5 3 L/min

Case 30 Man 44 with pericardial tumor

Roentgen data Left ventricular angiography demonstrated a diffuse expansive process within the pericardium roentgen diagnosis was pericardial tumor It was observed that the tip of the double lumen catheter was located in the sinus of Valsalva and restricted the motion of one of the aortic valve cusps The ascending aorta was short and wide $Q_m = 7\,4$ cm² $L = 7\,1$ cm Q_m varied by less than 5 per cent of the end diastolic value

Fick data
Oxygen consumption 242 cm³/min
A V O_2 diff 29 ml/L
Cardiac output 8 3 L/min
Heart rate 88 beats/min
Stroke volume 95 cm³

Differential pressure and flow

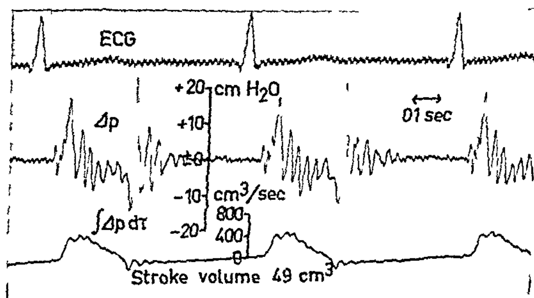


Fig 26 Calibrated curves for differential pressure and instantaneous blood flow

It will be observed that the flow curve drops below the end diastolic level at the end of systole — an observation verified by graphical integration The area enclosed by the flow curve above the end diastolic level corresponds to a stroke volume of 49 cm³ and the calculated cardiac output is 4 2 L/min

Case 33 Man 29 with mitral stenosis

Roentgen data Angiocardiography demonstrated mitral stenosis The aortic valve and the aorta were normal

$Q_m = 6\,6$ cm² $L = 7\,5$ cm Q_m varied by less than 6 per cent of the end diastolic value

Fick data Oxygen consumption 230 cm³ min
A V O_2 diff 29 ml/L
Cardiac ouput 8 0 L/min
Heart rate 75 beats min
Stroke volume 107 cm³

Differential pressure and flow

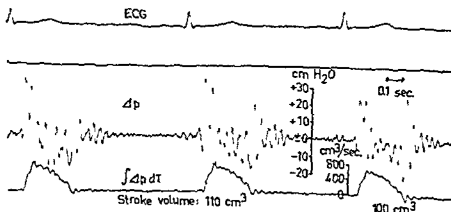


Fig 29 Calibrated curves for differential pressure and instantaneous blood flow

The mean flow varied during the Fick period as shown in Fig 30 The cardiac output obtained by averaging the flow *during the Fick* period was 7 9 L/min

Case 32 Woman, 32, with mitral stenosis *Digital valvulotomy had been performed five years earlier*

Roentgen data *Angiocardiography demonstrated mitral stenosis The left heart and the aorta were faintly opacified but there were no manifest abnormalities within the valve and the aorta*

$Q_m = 6\,0$ cm² $L = 7\,0$ cm It was not possible to measure the variations of Q_m

Fick data Oxygen consumption 178 cm³/min
A V O_2 diff 42 ml/L
Cardiac output 4 2 L/min
Heart rate 112 beats/min
Stroke volume 38 cm³

Differential pressure and flow

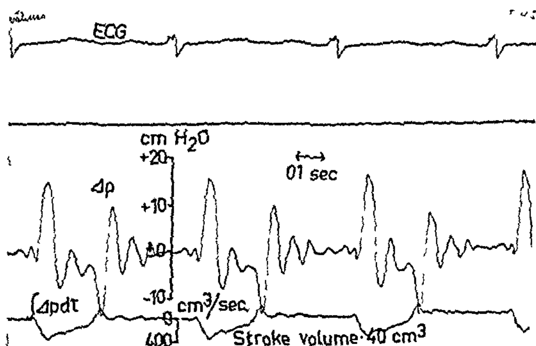


Fig 28 Calibrated curves for differential pressure and instantaneous blood flow See text

The symmetry of the system was tested in this case by reversing the catheter endings The mean flow was stable during the Fick period The calculated cardiac output was 4 3 L min

Case 33 Man, 29, with mitral stenosis

Roentgen data Angiocardiography demonstrated mitral stenosis The aortic valve and the aorta were normal

$Q_m \simeq 6\ 6\ cm^2$ $L \simeq 7\ 5\ cm$ Q_m varied by less than 6 per cent of the end diastolic value

Fick data Oxygen consumption 230 cm^3 min
A V O_2 diff 29 ml/L
Cardiac ouput 8 0 L/min
Heart rate 75 beats/min
Stroke volume 107 cm^3

Differential pressure and flow

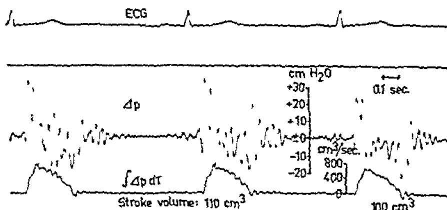


Fig 29 Calibrated curves for differential pressure and instantaneous blood flow

The mean flow varied during the Fick period as shown in Fig 30 The cardiac output obtained by averaging the flow during the Fick period was 7 9 L/min

Case 32 Woman, 32, with mitral stenosis Digital valvulotomy had been performed five years earlier

Roentgen data Angiocardiography demonstrated mitral stenosis The left heart and the aorta were faintly opacified but there were no manifest abnormalities within the valve and the aorta

$Q_m = 6.0$ cm² $L = 7.0$ cm It was not possible to measure the variations of Q_m

Fick data Oxygen consumption 178 cm³/min
A V O_2 diff 42 ml/L
Cardiac output 4 2 L/min
Heart rate 112 beats/min
Stroke volume 38 cm³

Differential pressure and flow

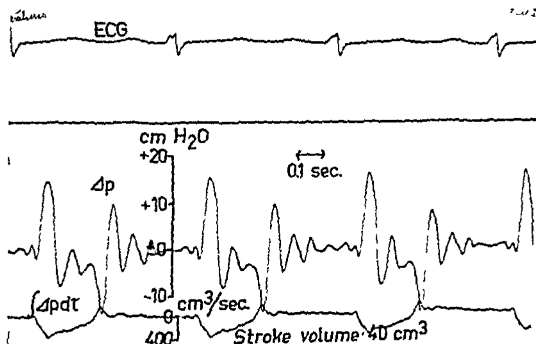


Fig 28 Calibrated curves for differential pressure and instantaneous blood flow See text

The symmetry of the system was tested in this case by reversing the catheter endings The mean flow was stable during the Fick period The calculated cardiac output was 4 3 L/min

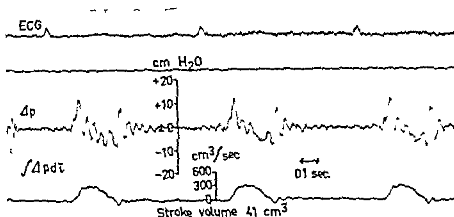


Fig 31 Calibrated curves for differential pressure and instantaneous blood flow (see text)

Prior to examination the patient had sinus rhythm, but the heart catheterization gave rise to flutter of a few minutes duration, after which sinus rhythm returned. A transient blood pressure fall had also occurred immediately before angiography.

Differential pressure and flow. Since the cross sectional area of the aorta could *not* be determined, it was not possible to quantitate the wave form of flow. The scale

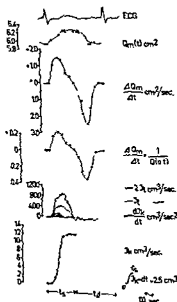


Fig 32 Case 31 Graphical calculation of the correctional term in eq (5) See text

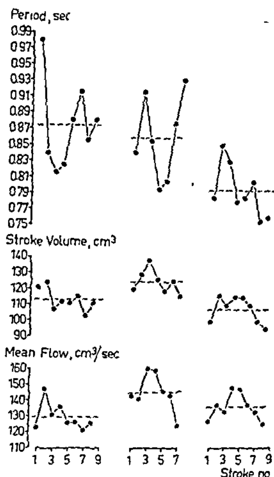


Fig 30 Case 33 Period (heart cycle duration), stroke volume and mean flow of 9, 7 and 9 consecutive hearts beats from early mid and late phases of the Fick period Broken lines indicated mean values

Case 34 Woman, 54, with *mitral stenosis*

Roentgen data *At angiocardiography the left side of the heart and the aorta were not opacified*

L approximately 7 0 cm

Fick data Oxygen consumption 178 cm^3/min
A-V O_2 diff 62 ml/L
Cardiac output 2 9 L/min
Heart rate 82 beats/min
Stroke volume 35 cm^3

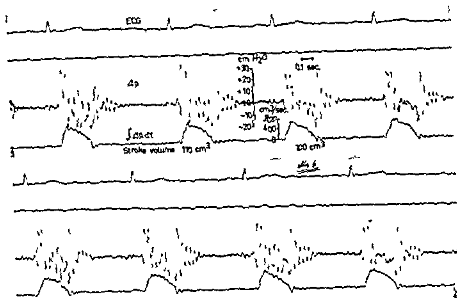


Fig 33 b These tracings were obtained immediately after those in Fig 33 a The integrator was switched on during diastole and the first flow curve starts from the level set fort zero flow As integration proceeds the baseline of the flow curves deviates from zero level, an effect which is not detectable during the first four heart beats

calculated from the correctional term The liquid volume arrived at by integration of I_k from the end of diastole ($t=0$) to the end of systole ($t=t_s$) must be added to the stroke volume to correct for the elastic deformation of the vessel In this case the correction will be 3 3 per cent of the stroke volume

Flow Curves

In series of flow curves from consecutive heart beats, the flow, as shown in Fig 33, conforms to a regular pattern and the diastolic level exhibits no systematic deviation from the level set for zero flow It will be seen from these records, taken during rest that the stroke volume varies slightly An abrupt change in the left ventricular output was registered when the patients performed the Valsalva manœuvre Records from such an experiment are shown in Fig 34 For calibration of the flow curves it was assumed that the cross sectional area of the aorta was unaffected by the experiment

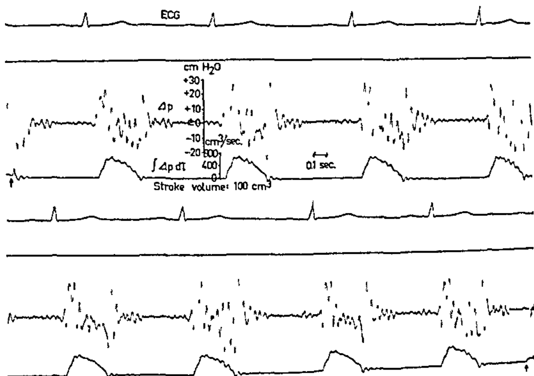


Fig 33 a The records above are referable to eight consecutive heart beats Integration starts to the left on the top tracing (indicated by a transient deflection on the differential pressure record) and terminates to the right on the second one There is no significant alteration of the baselines

of the flow curve shown below was obtained by giving Q_m/L, arbitrarily, a value of unity

The stroke volume calculated from the flow curve quantitated by using a tentative constant is found to be 41 cm³, equivalent to a cardiac output of 3 4 L/min

Cross sectional Area of the Ascending Aorta

In the few cases studied here the mean cross sectional area of the ascending aorta ranged between 5 9 and 7 4 cm² It will be seen from formula (5) that the area was measured at $t=0$, i e, at the end of diastole The variations of Q_m during the heart cycle were less than 7 per cent of the end diastolic value In patient No 31 who showed the greatest variations of cross sectional area, it was possible to plot Q_m as a function of time From the resulting curve the correctional term in eq (5) was calculated graphically The various steps involved in this calculation are shown in Fig 32 In the diagram, I_l denotes the flow $Q_m(0) \cdot U(t)$ and I_k the flow

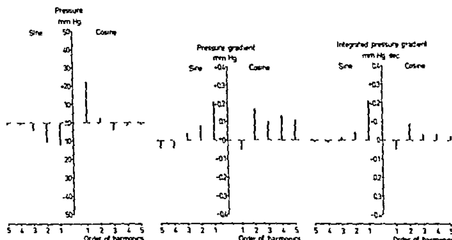


Fig 36 Case 29 Frequency spectra of ascending aortic pulse pressure pressure gradient and integrated pressure gradient

Potential and Kinetic Energy

The potential energy delivered by the left ventricle to the aorta was calculated graphically from pressure and flow curves recorded at brief intervals Pressure, flow, and heart rate showed no appreciable alteration during the period required for the recordings Instantaneous values for potential energy per unit time are plotted against time in cases 28 29 32 and 33 In case 28 values for kinetic energy per unit time are given for comparison The potential energy per heart beat, calculated from mean flow and mean pressure is denoted by W_m and the value determined from formula (9) by $W(T)$ It will be seen from Table III that $W(T)$ is higher than W_m in cases 28 29 and 33 and lower in case 32 The kinetic energy calculated from mean velocity is denoted by E_m and the value determined from formula (10) by $E(T)$ E_m is found to be much smaller than $E(T)$ in all cases The kinetic energy per heart beat, $E(T)$ is 1 7 per cent of the total energy in case 33 and 0 5 per cent of that energy in case 29 The kinetic energy was also calculated *ad modum* Prec, Katz, Sennet Roseman Fishman and Hwang (1949) from the formula $M v^2/2g$ where M is the stroke volume times the gravity of blood and v the velocity of flow obtained by dividing the stroke volume by the duration of the ejection and the cross sectional area of the aorta g is the gravitational constant The kinetic energy determined in this manner is denoted by $E+$ It will be seen that $E+$ is 40—60 per cent lower than $E(T)$ in these cases

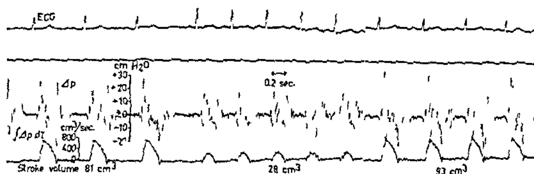


Fig 34 Records illustrating the effect of the Valsalva manœuvre upon the ascending aortic blood flow The tracings are cut from a continuous record begun just before strain (indicated on extreme left by a transient deflection of the differential pressure curve) The middle tracing was taken during strain and the right tracing just after the termination of strain

Frequency Components of Aortic Pulse Pressure and Pressure Gradient

Analysis of pulse pressure and pressure gradient for five harmonics was performed in cases 28 and 29 The amplitudes of the sine and cosine terms in the Fourier series representing the pulse pressure are shown in Figs 35 and 36 The amplitude of harmonics higher than the third is seen to be very small as compared to the fundamental frequency For the pressure gradient in each figure the amplitude of higher harmonics is of considerable magnitude The frequency spectra obtained by integrating the pressure gradient many be seen in Figs 35 and 36 The amplitude of the higher harmonics though reduced is not inappreciable as compared to the fundamental frequency

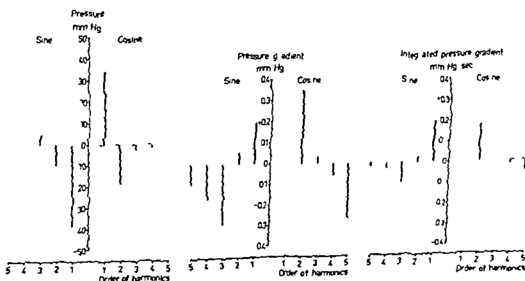


Fig 35 Case 28 Frequency spectra of ascending aortic pulse pressure pressure gradient and integrated pressure gradient

GENERAL DISCUSSION

A model circulatory system was constructed for the purpose of simulating the motion of blood in the proximal aorta (Porje and Rudewald, 1961). The instantaneous flow values obtained by the differential pressure method were in close accord with those yielded by the model. These model experiments were designed to meet the requirements laid down in the theory. Their significance derives from their affirmation that the method here applied to a biological system is founded on physically sound assumptions.

Some comments concerning the conditions requisite for application of the theory to the motion of blood in the human ascending aorta are first in order here. The equations valid for non-viscous flow have been used for the calculation of ascending aortic blood flow. This approach is, at the present time, justifiable since precise knowledge on the nature of the blood flow in the proximal aorta is lacking and appropriate corrections for viscous flow in an elastic tube as given by Womersley are impracticable. Furthermore the limitations of the present apparatus will render questionable any corrections for the effect of viscosity, as will be subsequently discussed. In theory the ascending aorta is regarded as a straight, elastic walled, branchless cylindrical tube. Although the vessel is usually slightly curved both in the lateral and the frontal projection the centripetal forces resulting from the curvature of the vessel are negligible as compared to those acting in the axial direction. The cross-section of the ascending aorta is probably circular under normal conditions, but deformation of the vessel could occur under the influence of certain factors, e.g. low intravascular pressure or increased intrathoracic pressure. In the course of this work it was possible to study the effect of the elastic deformation of the ascending aorta. The relevant corrections, computed according to the theory, show the effect to be of secondary importance. That portion of the ascending aorta which lies between the ostias of the coronary arteries and the origin of the innominate artery satisfies the requirement of a branchless tube. To determine the constant of integration in equation (6) it is assumed that the blood in the proximal aorta is at rest on termination of diastole. Although this is not strictly the case even when the aortic valve is normal — due to the coronary flow — the error introduced by

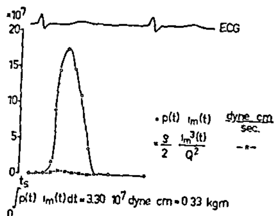


Fig 37 Case 28 Potential and kinetic energy per unit time

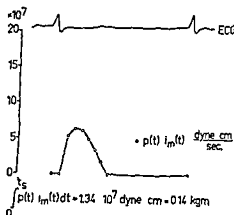


Fig 38 Case 29 Potential energy per unit time

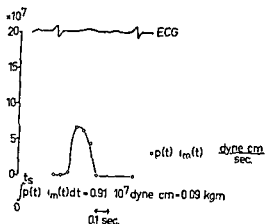


Fig 39 Case 32 Potential energy per unit time

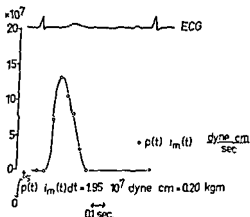


Fig 40 Case 33 Potential energy per unit time

Table III

Potential and kinetic energy per heart beat expressed in grammetres (GmM) p_m is the mean arterial pressure in mm of mercury See page 53

Case No	*W(T) GmM*	*W_m GmM*	*E(T) GmM*	*E_m GmM*	*E+ GmM*	*Cardiac output L/min*	*p_m mm Hg*
28	330	250	4 5	0 27	2 2	10 3	157
29	140	120	0 7	0 04	0 4	4 4	114
32	90	100	0 5	0 03	0 2	4 3	191
33	200	170	3 4	0 18	2 0	7 9	129

were the same during measurement of differential pressure and cardiac output as at angiography The angiographic examination was performed under general anaesthesia and a slight elevation of intrapulmonary pressure was manifest during the examination The mean arterial pressure remained stable throughout the exami nation except in case 34, where a marked fall of the blood pressure was recorded In the remaining cases the experimental error introduced by the relevant assumption should be of minor importance In four cases the cardiac output measured by the differential pressure method was found to be within ±20 percent of the value deter mined by the Fick method The cardiac output ranged from 4 2 to 8 0 L/min and the arterial pressure from 114 to 191 mm Hg in these cases In one case (No 28) the cardiac output calculated from the differential pressure curve was 34 per cent higher and in another case (No 30) 50 per cent lower than the corresponding Fick value In case 28 the mean flow changed during the Fick period, suggesting that the patient was not in a steady state — a circumstance which could have accounted for the deviation of the results The position of the catheter with the distal opening above the origin of the innominate artery could also have influenced the results In case 30 angiography disclosed that the catheter tip was situated in the sinus of Valsalva where it interfered with the movements of one of the aortic cusps The apparent instantaneous flow calculated from the differential pressure record drops below the end diastolic value at the end of systole and then climbs during diastole Although such a curve is indicative of valvular insufficiency, this condition was ruled out at angiography The apparent stroke volume calculated from the area enclosed by the flow curve above the end diastolic level, was much lower than that determined by the Fick method Similar behaviour was observed in case 31, where the catheter was close to the valve though here the stroke volume was reasonably consonant with the Fick value These observations reinforce those made when the catheter was withdrawn from the left ventricle to the aorta A catheter position close to the valve will result in distortion of the differential pressure wave form The flow curve calculated from such a differential pressure curve will be negative during diastole — a highly improbable result for subjects with intact aortic valves It must be concluded, therefore that the aortic region close to the valve should be avoided in the deter mination of differential pressure The two methods used for measuring mean flow are based on different physical assumptions and entirely different cond...

... as determined by the differential pressure method falls within 15—20 per cent of the Fick value, such a result should be satisfactory with respect to the accuracy of the measurements performed Theoretical appraisal led to the conclusion that the physical assumptions on which the differential pressure method is founded are largely valid when applied to the motion of blood in the human ascending aorta The experimental results obtained here support this

this zero condition will probably be small except in cases with valvular insufficiency or high heart rates From these theoretical considerations it is inferred that the equations used in this work are serviceable approximations for computing the flow in the human ascending aorta

The calculation of instantaneous blood flow and other hemodynamic data is based on a measured pressure difference The development of apparatus for this purpose initiated by Porjé and Rudewald in 1955, has been a time consuming and often exacting task Dynamic and static calibration of the catheter manometer system devised for this work shows that the performance of the system is adequate for the present purpose, as stated previously Future work in this field, however, would benefit from an improved frequency response of the system as well as from an arrangement for simultaneous recording of pressure and differential pressure The percutaneous technique for introduction of the double lumen catheter into the arterial system has proved safe and is, for a person with some experience of arterial puncture, a simple procedure For successful recording of differential pressure the catheter manometer system must be carefully prepared and meticulous precautions taken to exclude air bubbles from the system

The differential pressure curve recorded in the ascending aorta will represent the wave form of instantaneous blood acceleration Even this curve may provide useful information, as will be shown further on The curve obtained by integration of the differential pressure will represent the wave form of instantaneous blood velocity and flow To quantitate the wave forms of acceleration and velocity the density of blood and the distance between the catheter side holes must be determined Approximate values for these parameters are easily obtained For the determination of instantaneous blood flow it is also necessary to measure the cross sectional area of the ascending aorta At the present time this can be performed in humans only with the aid of advanced radiological methods By further integration of the flow curve the stroke volume as well as the mean flow can be determined Flow measurements via the differential pressure method is at the present time a formidable procedure, involving as it does angiographic determination of the cross sectional area of the aorta Sufficient data for determination of flow will be available, therefore, only in cases where the ascending aorta is visualized at diagnostic angiography A simplified technique for determination of the cross sectional area of the aorta would obviously facilitate the flow measurements

Measurements of the instantaneous ascending aortic blood flow in intact man have not been previously performed and it is not possible, therefore to compare the results with corresponding ones from another technique The mean blood flow as calculated by the differential pressure method can, however, be compared to that determined by conventional methods In the present work, values for cardiac output as determined via the Fick method are used for comparison To determine the flow from the differential pressure it was necessary to assume that the aortic dimensions

tion defined on page 37 tend to decrease with increasing age There also appears to be some sex difference The series is too small to permit conclusions and the results might have been influenced by the selection of the material It is fascinating to speculate about the possible correlation between the acceleration of the ascending aortic blood flow and the mechanical properties of the left ventricular myocardium Experimental investigations on dogs by Olsson Gimenez, Stauffer Carter and Lynch (1961) suggest that such a correlation exists Using high speed cinefluorography they found that the attainment of peak velocity in the ascending aorta was considerably delayed in animals with experimentally induced myocardial infarction Their data suggest that the mean acceleration during the period between the onset of the first heart sound and the attainment of maximum velocity is reduced after myocardial damage Arvidsson (1961) measured the left ventricular volume by rapid serial angiocardiography and was able in a few cases to determine the acceleration during left ventricular systole Maximum acceleration values of 2 and 3 *g* were obtained In this series the maximum acceleration ranged between 0 7 and 2 9 *g*

The hemodynamic data derived from this study should be of particular relevance as regards the design and development of future equipment in this field Ideally the differential pressure should be determined over a distance of infinitesimal length Such measurement however is beyond the capacity of any apparatus and it is therefore necessary to average the differential pressure over a finite interval of sufficient length to give a differential pressure signal consistent with the sensitivity of the pressure sensing device The error stemming from Pitot effects will be reduced by increasing the distance between the measuring points On the other hand that distance should not exceed the length of the ascending aorta between the level of the ostia of the coronary arteries and the origin of the innominate artery This length is subject to individual variation and it is necessary to ascertain empirically the interval at which the catheter side holes should be spaced to fit the ascending aorta without reducing the differential pressure signal below a practical limit In the cases studied here the distance available for the differential pressure measurement ranged between 7 and 9 cm but there are cases in wh ch the corresponding distance is only about 5 cm The maximum value of the pressure gradient ranged between 0 5 and 3 1 cm H_2O per cm Assuming for instance that the effective distance is put at 5 cm the maximum d fferential pressure would then be 2 5 cm H_2O Recording of signals of that order of magnitude with full scale deflection makes great demands on the performance of the differential pressure transducer as well as on the stability and gain of the amplifying network

Reliable values for the cross sect onal area of the ascending aorta in intact man are attainable at the present time only with the aid of radiology Utilization of the aortic diameter values collected by Suter (1897) from autopsy material would hardly be feasible since his figures are much lower than those recorded in living humans No systematic studies of the d mensions of the ascending aorta in intact man have

conclusion As techniques improve it should be possible to define more closely the limitations of the theoretical approach used in this study The flow curves calculated from the theory are plausible Their details, however, might be modified by application of the Womersley equation Although resonance oscillations resulting from catheter vibrations cause minor distortions of the flow curve, their net effect is nominal

Proceeding now to a more detailed discussion of some results, the pattern of the flow curves derived here is, for reasons implicit in the theory, identical with that of the velocity curve Such curves have been published earlier by Porjé and Rudewald (1957, 1960 and 1961) and are similar to those derived by Barnett *et al* (1961), though the technique differs in certain respects The latter authors pass the differential pressure signal through an analogue computer adjusted to compensate for the blood viscosity The flow curves obtained here, on the other hand, are the result of integration of the differential pressure signal, the effects of viscosity being disregarded for reasons already discussed Of particular interest in this context are the flow curves shown in fig 33, since the position of their base line in relation to a pre set level for zero flow is subject to the influence of e g viscosity Thus the pressure fall caused by the steady flow component would result in a D C signal from the differential pressure transducer The integral of that signal would be a line deviating in a positive direction from the pre set level for zero differential pressure — viz zero flow — and the flow curves would be superimposed on that line By integration during series of successive heart beats it would thus be possible to measure even a small steady pressure drop from which the steady flow could be calculated via the Poiseuille equation The mean pressure drop due to viscosity, as calculated for case 33, was 0 016 cm H_2O With the sensitivity setting used in this case integration for seven seconds would result in a positive deflection of about 2 mm Accurate measurement of signals of that magnitude requires very high stability and sensitivity of the recording system and is not possible with the present apparatus, as shown by Fig 33 Particular measures are required, moreover, to ensure that the catheter openings are always in the horizontal plane Otherwise there will be recorded a static differential pressure equal to the difference between the specific gravities of blood and saline multiplied by the vertical distance between the catheter openings If that distance is approximately a quarter of a centimetre the static pressure difference will be of the same order of magnitude as the pressure fall due to viscosity Thus to obtain information about the effect of viscosity from the position of the base line of the flow curves it will be necessary to take this effect into account

As pointed out above the differential pressure curve represents the wave form of instantaneous acceleration — or more precisely the mean instantaneous blood acceleration within a portion of the ascending aorta of length *L* The differential pressure method thus affords possibilities of studying in human beings hitherto unknown properties of the blood flow in the vicinity of the heart Values for mean accelera

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SUMMARY

The theoretical conditions relevant to determination of instantaneous ascending aortic blood flow from a measured pressure gradient are explored

The performance of the catheter manometer system used for the measurement of differential pressure is analyzed

The technique for measuring the differential pressure in the human ascending aorta is described in detail

Data on the hemodynamics of the ascending aorta e g pressure gradient and instantaneous values for acceleration velocity and flow as derived from measurements in a series of patients are set forth

The mean flow determined by the differential pressure method is compared to that obtained by the Fick method in seven cases in the majority of which good agreement is shown It is concluded that the physical assumptions on which the differential pressure method is founded are largely valid when applied to the motion of blood in the human ascending aorta.

The clinical application of the derived information concerning the acceleration of blood in the ascending aorta is discussed

Potential and kinetic energy delivered by the heart to the aorta is calculated, in a few cases from instantaneous values for pressure and flow

The limitations of the present apparatus are defined and data which could provide guidance for the elaboration of apparatus in this field are presented

apparently been published to date If a close correlation were demonstrable between the cross sectional area of the ascending aorta and, for example, age, sex, and body surface area, it might provide a basis for plausible predictions of the first metioned area, thereby simplifying the determination of instantaneous blood flow Studies of angiographic material are now being pursued by Sténson at the Roentgen Department I Sodersjukhuset In the cases studied here the mean cross sectional area of the ascending aorta ranged between 5 9 and 7 4 cm² The effective distance, measured in centimeters was of roughly the same order of magnitude It is interesting to note that in case 34 the assumption that $Q_m/L=1$ gives a cardiac output close to that obtained by the Fick method

The mechanical energy delivered by the left ventricle to the aorta has been calculated from instantaneous values for pressure and flow in a few cases Because of the limitations of previously available methods no such calculations were earlier possible in man Katz (1932) showed that, in the turtle heart, the use of mean pressure and mean velocity for calculating the external work of the heart resulted in underestimation of kinetic and potential energy and altered the calculated distribution of the work between these two forms Prec *et al* (1949) studied the kinetic energy of the human heart by cardiac catheterization and angiocardiography They calculated the potential energy from the stroke volume and the mean pressure in the aorta The kinetic energy in the aorta was calculated from a formula involving the velocity of flow obtained by dividing the stroke output by duration of ejection and the cross sectional area of the aorta It was pointed out that the data obtained from their formulae may underestimate, in particular, the energy expended by the ventricle in imparting velocity to the blood — The potential energy calculated from the general formula (9) is considerably higher than the corresponding value determined from mean values of pressure and flow in one case (No 28) The results for cases 29 and 33 likewise suggest that the potential energy calculated from mean values is underestimated In the case of kinetic energy the underestimation is very pronounced Values for kinetic energy computed *ad modum* Prec *et al* are found to be 40—60 per cent lower than those obtained from equation (10) If the mean velocity is used for the calculation the kinetic energy will have a much *lower value* than that obtained by integration The kinetic energy constitutes 0 5 to 1 7 per cent of the total energy in these cases The same proportions were obtained by Prec *et al* Graphical calculation of potential energy from instantaneous values for pressure and flow is tedious and not very accurate For more accurate *calculation of potential* energy simultaneous recording of pressure and flow is required Electrical multiplication of the signals and the use of automatic data processing *would, of course,* greatly facilitate the calculations

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LIST OF NOTATIONS

x	Distance measured along the axis of the tube (the ascending aorta)
L	Distance between pressure measuring points along the axis of the tube
r	Distance from the centre of the cross-section of the tube
R	Radius of the cross section of the tube
t	Time
$p(x, t)$	Pressure
Δp	Differential pressure between measuring points at $x=0$ and $x=L$
$\Delta p/L$	Pressure gradient
$Q(x, t)$	Cross sectional area
$Q_m(t)$	Mean cross sectional area of a portion of the ascending aorta between $x=0$ and $x=L$ defined by $$Q_m(t) = \frac{1}{L}\int_0^l Q(x,t)\ dx$$
$v(x, t)$	Velocity of blood in the axial direction
$U(t)$	Mean instantaneous blood velocity defined by $$U(t)= \frac{1}{L}\int_0^l v(x,t)\ dx$$
$i(x, t)$	Volume flow
$i_m(t)$	Mean instantaneous flow defined by $$i_m(t)= \frac{1}{L}\int_0^l i(x, t)\ dx$$
$a(t)$	Mean instantaneous acceleration defined by $$a(t)=\frac{dU(t)}{dt}$$
$W(t)$	Potential energy
$W(T)$	Potential energy per heart beat
W_m	Potential energy per heart beat calculated from mean values for pressure and flow
$E(t)$	Kinetic energy
$E(T)$	Kinetic energy per heart beat
E_m	Kinetic energy per heart beat calculated from mean velocity
α	Parameter defined by $$\alpha = R\sqrt{\frac{\omega}{\nu}}$$
γ	Friction constant
ρ	Density
ν	Kinematic viscosity
ω	Circular frequency
ω_R	Resonance circular frequency
f	Frequency

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Contribution of Alveolar Ventilation, Pulmonary Blood Flow and Venous Gas Concentrations to Oxygen Uptake and *Carbon Dioxide Output*

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Introduction

In the steady state the rates of oxygen uptake and carbon dioxide output through the lungs must be sufficient to satisfy the metabolic needs of the body An adjustment of the rates to varying metabolic needs can be achieved by appropriate changes in one or more of the following variables

(1) Alveolar ventilation
(2) Pulmonary capillary blood flow
(3) Gas concentrations in mixed venous blood

If gas tensions of inspired air and gas combining capacities of arterial blood are given the maximum rates of gas exchange which can be attained will be completely determined by these variables A better understanding of the mechanisms by which the body can meet increased metabolic needs under various conditions may therefore be obtained by considering the rates of gas exchange as functions of these variables Under certain simplifying assumptions which will be specified below such functions can be derived analytically from equations and biochemical relations which are commonly used in respiratory physiology The mathematical derivation of these functions is given in an Appendix to the present paper The two following sections will give the principal assumptions on which this derivation is based and a general discussion of the results based on numerical examples and graphical illustrations

Basic assumptions used in the analysis

To carry out the analysis it was necessary to make the following assumptions

Graphical demonstration and discussion of some principal results of the analysis

The relations developed in the Appendix will be illustrated graphically in co-ordinate systems where alveolar ventilation is plotted along the abscissa and pulmonary capillary blood flow along the ordinate The gas concentrations in mixed venous blood and in inspired air are assumed to be constant for each graph and the barometric pressure to be 760 mm Hg in all graphs Each point with positive abscissa and ordinate values will then represent a given combination of alveolar ventilation and pulmonary blood flow and points which yield equal rates of gas exchange will be connected by curves It follows from the relations developed in the Appendix that all these curves will be hyperbolas with asymptotes parallel to the co-ordinate axes The distances between the axes and the asymptotes will generally increase with increasing rates of gas exchange provided that other conditions are kept constant

Curves representing four rates of oxygen uptake from 250 to 400 ml/min are shown in each of the six graphs given in figure 1a The venous oxygen concentration is assumed to be 15 vol per cent in the three graphs to the left and 12 vol per cent in the three graphs to the right while the oxygen fraction in inspired air is 0 14 in the two upper 0 21 in the two middle and 1 00 in the two lower graphs In all six graphs the hemoglobin content of the blood is assumed to be 15 gram/100 ml and the respiratory quotient 0 8 A line indicating the average normal ventilation blood flow ratio $\dot{V}_A/\dot{Q}_c = 0\ 8$ and a point N indicating the approximate normal rest values for adults $\dot{V}_A = 4400$ ml min and $\dot{Q}_c = 5500$ ml/min are inserted in each of the six graphs

The second graph from above in the left column illustrates curves when ordinary atmospheric air is inspired and the mixed venous blood contains 15 vol per cent oxygen The location of point N near the lowest curve indicates that under these conditions an oxygen uptake of 250 ml/min should be *theoretically* possible with an alveolar ventilation of 4400 ml/min and a pulmonary blood flow of 5500 ml/min This result of the theoretical analysis fits very well with actually observed values in normal adults during rest It may perhaps be taken as an indication that

(1) A constant ventilation perfusion ratio throughout the lungs (or in the part of the lungs which is considered in the analysis)

(2) Negligible alveolo capillary tension differences for oxygen as well as for carbon dioxide

(3) Identical gas tensions in mixed end pulmonary capillary blood and in arterial blood i e a negligible physiological shunt

It is well known that such ideal conditions do not exist in any lung In persons without pulmonary diseases the deviations from these conditions are probably small and can be disregarded for the present purpose at least as a first approximation Great deviations from the ideal conditions may however be found in patients with lung diseases particularly with emphysema or fibrosis

It should be emphasized therefore that the analytical relations which have been developed yield *maximum rates* of gas exchange i e rates which could be obtained with given values of the three variables under otherwise ideal conditions for gas exchange between inspired air and blood Actual rates will be smaller than these theoretically calculated rates if there is an unequal distribution of ventilation and blood flow impaired diffusion or a *marked venous arterial shunt*

Even in cases with a varying ventilation blood flow ratio the relations developed for ideal conditions may be applied to any limited part of the lungs in which the ventilation blood flow ratio can be regarded as constant In a subsequent analysis of certain effects of uneven distribution of ventilation and blood flow the relations will be applied to small and presumably homogeneous *subdivisions of the lungs*

For each gas three equations were used to develop analytical relations between rates of gas exchange alveolar ventilation pulmonary blood flow and venous gas concentrations Two of these equations are well known and generally referred to as the alveolar ventilation equation and Fick s equation The purpose of the third equation was to relate gas concentrations to gas tensions in *arterial blood As shown in the Appendix* close fits to the experimentally determined relationship between these two quantities can be obtained by second order *equations with appro*priately chosen constants which will depend on the gas combining capacities of the blood

the ideal conditions on which the analysis was based, are nearly fulfilled in such persons The other three curves illustrate the combinations of alveolar ventilation and pulmonary blood flow which are necessary for higher oxygen uptakes, when atmospheric air is inspired and a venous oxygen concentration of 0 15 shall be maintained

The second graph from above in the right column shows the combinations of alveolar ventilation and pulmonary blood flow which are necessary for the same four rates of oxygen uptake, when venous oxygen concentration is reduced to 0 12, and conditions otherwise are unchanged All curves have been shifted toward the co-ordinate axes, which means that the same oxygen uptake can now be achieved with lower ventilation and/or blood flow The location of point N indicates, for example, that an alveolar ventilation of 4400 ml/min and a blood flow of 5500 ml/min can give an oxygen uptake as high as 350 ml/min under these conditions It is seen that the curves have been shifted somewhat more toward the abscissa than toward the ordinate, which means that a reduction in venous oxygen concentration may give a greater relief for circulation than for ventilation

Within a narrow interval near the line $\dot{V}_A/\dot{Q}_c = 0\ 8$, the curves in both graphs indicate that an increase in pulmonary blood flow can compensate a decrease in alveolar ventilation, and vice versa, so that oxygen uptake (and venous oxygen concentration) can be kept on the same level For each rate of oxygen uptake (with specified concentrations of oxygen in inspired air and in venous blood) there are, however, certain minimum values of alveolar ventilation and pulmonary blood flow — defined by the distances between the two asymptotes of the curve and the two co ordinate axes A reduction of either ventilation or blood flow below the minimum value cannot be compensated by any increase of the *other* variable A decrease in the oxygen concentration of the venous blood will then represent the only possible way in which the subject can maintain the original rate of oxygen uptake — as may be seen by comparison of the curves in the left hand and right hand graph

The upper left hand graph shows that an oxygen uptake *of* 250 ml/min with only 14 per cent oxygen in inspired air would require very high rates of ventilation and blood flow, if the venous

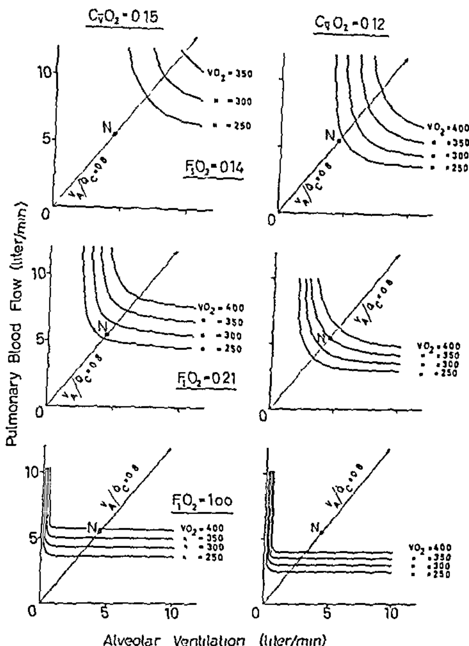


Figure 1 a

Curves showing combinations of alveolar ventilation and pulmonary blood flow which yield oxygen uptakes of specified sizes, for various oxygen concentrations in inspired air (F_IO_2) and in venous blood ($C_{\bar v}O_2$) (General assumptions Uniform ventilation blood flow ratio, no impairment of diffusion, no arterio venous shunt, barometric pressure 760 mm Hg, 15 gram Hb/100 ml blood, R Q = 0 8)

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oxygen concentration should be kept at 15 vol per cent An alveolar ventilation of 4400 ml/min and a blood flow of 5500 ml/min however will be almost sufficient for this oxygen uptake if venous oxygen concentration is reduced to 12 vol per cent as seen in the upper right hand graph

The two graphs at the bottom of figure 1a illustrate effects of inspiring pure oxygen The significance of alveolar ventilation for *the oxygen uptake is then almost completely eliminated* The *maximum rates of oxygen uptake which can be obtained under* these conditions will be determined essentially by the rate of blood flow and the venous oxygen concentration alone — provided that alveolar ventilation does not fall below extremely low values (less than 500 ml/min) It is quite obvious from these curves that inspiration of pure oxygen will be much more effective in the *correction of anoxemia caused by reduced ventilation than of one* caused by poor circulation

Generally it can be said that the distances between the co ordinate axes and the asymptotes of the curves increase in direct proportion to the rate of oxygen uptake but decrease with decreasing oxygen concentration in venous blood or with in creasing oxygen concentration in inspired air As shown in the *Appendix the distance between the ordinate and the vertical* asymptote (i e the minimum value of alveolar ventilation for a specified oxygen uptake) is approximately inversely proportional to the difference between the *oxygen tension* in inspired air and the oxygen tension in venous blood The distance between the abscissa and the *horizontal* asymptote (i e the minimum value of blood flow for a given oxygen uptake) on the other hand is approximately *inversely proportional to the difference between the* *oxygen concentration* in arterial blood exposed to inspired air and the oxygen concentration in venous blood

Curves representing four rates of *carbon dioxide output* from 200 to 350 ml/min are shown in similar co ordinate systems in the two graphs in figure 1b A *venous carbon dioxide concentration* of 50 vol per cent is assumed in the graph to the left and of 60 vol per cent in the graph to the right In both graphs it is assumed that inspired air contains no carbon dioxide and that arterial blood has a carbon dioxide combining power of 48 5 vol per cent at a tension of 40 mm Hg The line indicating $\dot{V}_A/\dot{Q}_c = 0.8$ and

the point N indicating $\dot V_A = 4400$ ml/min and $\dot Q_c = 5500$ ml/min are shown in both graphs

The curves resemble those found for oxygen, in so far as they are hyperbolas with asymptotes parallel to the axes, at distances

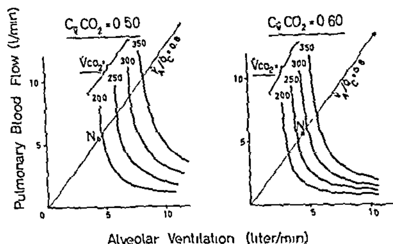


Figure 1 b

Curves showing combinations of alveolar ventilation and pulmonary blood flow which yield carbon dioxide outputs of specified sizes for two different carbon dioxide concentrations in venous blood ($C_{\bar v}CO_2$) (General assumptions Uniform ventilation blood flow ratio barometric pressure 760 mm Hg no carbon dioxide in inspired air carbon dioxide combining power of arterial blood = 48 5 vol per cent at p_aCO_2 = 40 mm Hg)

which increase with increasing rate of carbon dioxide output, and with decreasing carbon dioxide concentration in venous blood The position of point N in the graph to the left indicates that an alveolar ventilation of 4400 ml/min and a blood flow of 5500 ml/min should be theoretically sufficient for a carbon dioxide output slightly below 200 ml/min when venous carbon dioxide concentration is 0 50 — a result which is in accordance with actual observations

There are, however, also some differences between the curves for carbon dioxide and the curves for oxygen, particularly those obtained when pure oxygen is inspired (the two bottom graphs

in figure 1a) In contrast *to the oxygen uptake*, carbon dioxide output depends more on ventilation than on the rate of blood flow Moreover, an increase in venous carbon dioxide concentration causes a greater relief for ventilation than for circulation, which is the reverse of the effect of a reduced venous oxygen concentration

Considered together, the findings for oxygen and carbon dioxide imply *that a combined increase of alveolar ventilation and pulmonary* blood flow along the line $\dot{V}_A/\dot{Q}_c = 0\ 8$ in most cases is the most efficient way to meet increased metabolic needs This is particularly true when inspired air is composed of pure oxygen, but to some extent also when it contains ordinary atmospheric air An increase in ventilation, without changes in blood flow or venous gas concentrations, may enhance carbon dioxide output *considerably, with practically no effect on oxygen uptake* Conversely, an increase of blood flow alone may contribute considerably to the oxygen uptake, without affecting the carbon dioxide *output significantly A combined increase of ventilation and blood* flow along the line $\dot{V}_A/\dot{Q}_c = 0\ 8$ will give a proportionate increase of oxygen uptake and carbon dioxide output, with maintenance of a constant respiratory quotient

In diseases where either circulation or ventilation is impaired excessive changes in venous gas concentrations may be the result of increased metabolic demands Patients with heart diseases may, for example, compensate their reduced capacity to increase blood flow by increased ventilation This may be sufficient to provide for the increased carbon dioxide elimination, but not for the oxygen consumption, which can be increased only by a reduction *of the oxygen concentration* in venous blood In heart diseases with decreased cardiac output one should expect therefore that reduction of oxygen *concentration in venous blood would appear* sooner, and be more pronounced, than an increase *in* venous carbon dioxide concentration

Conversely, if alveolar ventilation is impaired, as in many pleural and pulmonary diseases, an increased cardiac output may

uptake but not of the

paired ventilation may

therefore, more often than heart diseases, lead to increased venous carbon dioxide concentrations

APPENDIX

The objective of the analysis is to relate (maximum) rates of gas exchange to alveolar ventilation, pulmonary blood flow and venous gas concentrations, under the previously stated ideal conditions for gas exchange between inspired air and blood. It is natural in these relations to consider alveolar ventilation, blood flow and venous gas concentrations as the independent variables, because they are the main quantities which the body can vary, at least within certain ranges, to adjust rates of gas exchange to metabolic needs. The rates of gas exchange may then be considered as functions of these three variables, and certain other quantities — composition of inspired air and gas combining capacities of arterial blood — as fixed parameters.

To avoid overloading of formulas and equations, it will be convenient, in the mathematical derivation of the relations, to replace the conventional standard symbols (Federation Proceedings, 9, 602—605. 1950) by denotations which are shorter and simpler. Defined in terms of the standard symbols the following denotations where chosen:

$\dot{V} = \dot{V}_A$, $\dot{Q} = \dot{Q}_c$ and R = respiratory quotient

$p = p_I O_2$	$P = P_B$
$f = F_I O_2$	
$x = p_A O_2 = p_a O_2$	$y = p_A CO_2 = p_a CO_2$
$\alpha(x) = C_a O_2$	$\beta(y) = C_a CO_2$
$a = C_{\bar{v}} O_2$	$b = C_{\bar{v}} CO_2$
$u = \dot{V} O_2$	$v = \dot{V} CO_2$

For each gas the relations were derived from three equations, the alveolar ventilation equation, Fick's equation, and an equation which relates the gas concentration of arterial blood to the gas tension.

For *oxygen*, the alveolar ventilation equation can be written as follows, in the abbreviated symbols:

$$u = \frac{273}{310}\left[\frac{p - x}{P}\right]\dot{V} + \frac{p(1 - P)}{(P - 47)}u \qquad (1a)$$

with u measured at *STPD* and $\dot{V}$ at *BTPS*. The second term on the right side accounts for the difference between inspired and expired alveolar volume.

Fick's equation reads

$$u = [a(x) - a]\dot{Q} \quad (2a)$$

Close fits to experimentally determined relations between oxygen tensions and oxygen concentrations in arterial blood can finally be obtained within a limited range of variation, by appropriate choices of the constants A, H and h, in an equation of the form

$$a(x) = A\left[\frac{x - H}{x - h}\right] \quad (3a)$$

In this equation, the oxygen concentration of arterial blood is considered as a function of the oxygen tension alone, the hemoglobin content being considered as a fixed parameter, which will influence the values of the constants (particularly the value of the constant A) Because of the Bohr effect, the oxygen concentration should, strictly speaking, be considered as a function of the oxygen as well as of the carbon dioxide tension If the respiratory quotient is constant, carbon dioxide tensions can be computed for each oxygen tension from the alveolar air equation, and the Bohr effect may then be taken into consideration in the relation between oxygen tension and concentration A numerical example, based on a hemoglobin content of 15 gram/100 ml blood and R $Q = 0$ 8, is given in table 1a The second column shows carbon dioxide tensions corresponding to the oxygen tensions given in the first column Observed oxyhemoglobin saturations in percent which

Table 1 a

Comparison of oxygen concentrations calculated from (*3 a*) using the values (*4 a*) for the constants and oxygen concentrations actually observed at various oxygen and carbon dioxide tensions with 15 gram hemoglobin per 100 ml of blood

p_aO_2 (mm Hg)	p_aCO_2 (mm Hg)	Per cent oxy Hb	C_aO_2 observ	C_aO_2 calc
50	83	75 0	0 152	0 150
60	75	84 0	0 170	0 170
70	68	90 0	0 182	0 182
80	59	93 0	0 189	0 189
90	51	95 5	0 194	0 194
100	41	97 5	0 198	0 198
110	34	98 5	0 200	0 201

are obtained from these tensions are shown in the third column. Corresponding oxygen concentrations — taking into account the physically dissolved oxygen as well as that combined with hemoglobin — are listed in column 4. The constants A, H and h were then determined so that the concentrations calculated from (3a) will *coincide with those given in column 4 at three points at* $p_A O_2 = 60$, 80 and 100 mm Hg. The following values were obtained:

$$A = 0.223 \qquad H = 32.5 \qquad h = 24 \tag{4a}$$

Calculated concentrations obtained when these values are used in (*3a*) are given in the last column of the table. Comparison of observed and calculated concentrations (columns 3 and 5) shows satisfactory agreement for all oxygen tensions between 50 and 110 mm Hg.

In cases where the respiratory quotient is unknown or variable (as may be the case in different parts of lungs with uneven ventilation in relation to blood flow), it is difficult to take the Bohr effect into consideration in the relation between oxygen tension and oxygen concentration. Equation (3*a*) may, however, be used with a fair degree of accuracy with the values

$$A = 0.222 \qquad H = 15 \qquad h = 4 \tag{5a}$$

if oxygen tension is above 60 mm Hg and carbon dioxide tension between 40 and 60 mm Hg. This is shown in table 2a, in which oxygen concentrations computed from (*3a*) — using the values (*5a*) for the constants — are compared with observed concentrations at various oxygen tensions *for carbon dioxide* tensions of 40, 50 or 60 mm Hg and a hemoglobin content of 15 gram/100 ml blood.

Elimination of $a(x)$ and x from (*1a*), (*2a*) and (*3a*) by straightforward arithmetic gives a general relation between u, V and Q which can be written in the form

$$(V - c_1 u)(Q - c_2 u) = c_3 u^2 \tag{6a}$$

where

$$\left.\begin{aligned} c_1 &= 1.13\,\frac{P(A - a)[1 - f(1 - R)]}{A(p - H) - a(p - h)} \\ c_2 &= \frac{(p - h)}{A(p - H) - a(p - h)} \\ c_3 &= 1.13\,\frac{PA[1 - f(1 - P)](H - h)}{[A(p - H) - a(p - h)]^2} \end{aligned}\right\} \tag{7a}$$

Fick's equation *reads*

$$u = [a(x) - a]\dot{Q} \qquad (2a)$$

Close fits to experimentally determined relations between oxygen tensions and oxygen concentrations in arterial blood can finally be obtained within a limited range of variation, by appropriate choices of the constants A, H and h, in an equation of the form

$$a(x) = A\left[\frac{x - H}{x - h}\right] \qquad (3a)$$

In this equation, the oxygen concentration of arterial blood is considered as a function of the oxygen tension alone, the hemoglobin content being considered as a fixed parameter, which will influence the values of the constants (particularly the value of the constant A). Because of the Bohr effect, the oxygen concentration should, strictly speaking, be considered as a function of the oxygen as well as of the carbon dioxide tension. If the respiratory quotient is constant, carbon dioxide tensions can be computed for each oxygen tension from the alveolar air equation, and the Bohr effect may then be taken into consideration in the relation between oxygen tension and concentration. A numerical example, based on a hemoglobin content of 15 gram/100 ml blood and $R/Q = 0.8$, is given in table 1a. The second column shows carbon dioxide tensions corresponding to the oxygen tensions given in the first column. Observed oxyhemoglobin saturations in percent which

Table 1 a

Comparison of oxygen concentrations calculated from (3a) using the values (4 a) for the constants and oxygen concentrations actually observed at various oxygen and carbon dioxide tensions with 15 gram hemoglobin per 100 ml of blood

p_aO_2 (mm Hg)	p_aCO_2 (mm Hg)	Per cent oxy Hb	C_aO_2 observ	C_aO_2 calc
50	83	75.0	0.152	0.150
60	75	84.0	0.170	0.170
70	68	90.0	0.182	0.182
80	59	93.0	0.189	0.189
90	51	95.5	0.194	0.194
100	41	97.5	0.198	0.198
110	34	98.5	0.200	0.201

effect due to different carbon dioxide tensions in arterial and venous blood is disregarded Nevertheless, we may interpret Δp roughly as the difference between the oxygen tension in inspired air and in mixed venous blood

The symbol Δa will be defined as the difference between the oxygen concentration computed from (3a) when $x = p$ and the oxygen concentration in venous blood i e

$$\Delta a = A \frac{(p - H)}{(p - h)} - a \tag{9a}$$

It follows from (9a) and (3a) that Δa may be interpreted as the difference between the oxygen concentration which would be obtained if arterial blood were exposed to inspired air, and the oxygen concentration in venous blood

If these symbols are used in (7a) c_1 and c_2 may be expressed as follows

$$\left.\begin{aligned} c_1 &= 1\ 13\,[1 - f(1 - R)]\frac{P}{\Delta p} \approx \text{approx } \frac{P}{\Delta p} \\ c_2 &= \frac{1}{\Delta a} \end{aligned}\right\} \tag{10 a}$$

From the first of these formulas it is seen that for a given rate of oxygen uptake the distance between the vertical asymptote and the ordinate $-c_1u-$ will be approximately inversely proportional to the oxygen tension gradient between *inspired air and venous* blood This distance indicates the theoretical minimum value of alveolar ventilation

— will be inversely *proportional* to the difference between oxygen concentration in arterial blood exposed to inspired air and oxygen concentration in venous blood This distance indicates the *theoretical minimum* value of blood flow which is necessary to maintain the particular oxygen uptake under the specified conditions

From these results and from the *shape* of the oxy hemoglobin curve it can be inferred that an increase of the

mu

mu

blood flow Conversely a reduction

Table 2 a

Comparison of oxygen concentrations calculated from (3 a) using the values (5 a) for the constants and oxygen concentrations actually observed at various oxygen tensions carbon dioxide tensions of 40 50 and 60 mm Hg and 15 gram hemoglobin per 100 ml of blood

p_aO_2 (mm Hg)	C_aO_2 observed when p_aCO_2 is			C_aO_2 calc
	40 mm Hg	50 mm Hg	60 mm Hg	
60	0 182	0 178	0 176	0 178
70	0 188	0 185	0 184	0 185
80	0 192	0 190	0 188	0 190
90	0 196	0 195	0 193	0 194
100	0 198	0 197	0 196	0 197
110	0 200	0 199	0 198	0 199

A simple graphical presentation of the relation (6a) can be given in co ordinate systems where alveolar ventilation is plotted along the abscissa and pulmonary blood flow along the ordinate If variables and parameters included in the three factors c_0 c_1 and c_2 are kept constant, points representing equal rates of oxygen uptake will then yield hyperbolas with asymptotes parallel to the co ordinate axes The distance between the vertical asymptote and the ordinate is c_1u and the distance between the horizontal asymptote and the abscissa is c_2u, i e both distances will in crease with increasing rate of oxygen uptake, if the two factors c_1 and c_2 are kept constant The significance of these two factors can be seen more easily by introducing two new quantities, Δp and Δa, defined as follows

Let Δp denote the difference between the oxygen tension in inspired air and the oxygen tension which can be computed from (3a) when $a(x) = a$, i e

$$\Delta p = p - \left[\frac{AH - ah}{A - a}\right] \quad (8a)$$

The first term on the right side of (8a) is the oxygen tension in inspired air and the second term is approximately equal to the oxygen tension in the venous blood This last statement is not quite correct, partly because the approximation given by (3a) is less accurate at low oxygen tensions, and partly because the Bohr

effect due to different carbon dioxide tensions in arterial and venous blood is disregarded Nevertheless we may interpret Δp roughly as the difference between the oxygen tension in inspired air and in mixed venous blood

The symbol Δa will be defined as the difference between the oxygen concentration computed from (3a) when $x = p$ and the oxygen concentration in venous blood i e

$$\Delta a = A \frac{(p - H)}{(p - h)} - a \qquad (9a)$$

It follows from (9a) and (3a) that Δa may be interpreted as the difference between the oxygen concentration which would be obtained if arterial blood were exposed to inspired air, and the oxygen concentration in venous blood

If these symbols are used in (7a) c_1 and c_2 may be expressed as follows

$$\left.\begin{aligned} c_1 &\sim 1\ 13\{1 - f(1 \quad R)\} \frac{P}{\Delta p} = \text{approx } \frac{P}{\Delta p} \\ c_2 &= \frac{1}{\Delta a} \end{aligned}\right\} \qquad (10\ a)$$

From the first of these formulas it is seen that for a given rate of oxygen uptake the distance between the vertical asymptote and the ordinate — $c_1 u$ — will be approximately inversely proportional to the oxygen tension gradient between inspired air and venous blood This distance indicates the theoretical minimum val e of alveolar –

$c_2 u$ — will be inversely proportional to the difference between oxygen concentration in arterial blood exposed to inspired air and oxygen concentration in venous blood This distance indicates the theoretical minimum value of blood flow which is necessary to maintain the particular oxygen uptake under the specified conditions

From these results and from the shape of the oxy hemoglobin curve it can be inferred that an increase of the oxygen tension in inspired air above 150 mm Hg will cause great reductions in mini mum values of ventilation but relatively small reductions in minimum values of pulmonary blood flow Conversely, a reduction

of venous oxygen concentration below the normal level of 15 vol per cent will have a considerably greater effect on the minimum *value of blood flow* than on the minimum value of ventilation

In the graphical illustrations of (*6a*) in figure 1a the barometric pressure was assumed to be 760 mm Hg the values 0 15 and 0 12 were chosen for the oxygen concentrations in venous blood and the values 0 14 0 21 and 1 00 for the oxygen fractions in inspired air The following numerical values were obtained for c_1 c_2 and c_0 under these assumptions and used for the curves in *figure* 1a

C_vO_2	F_IO_2	c_1	c_2	c_0
0 15	0 14	16 9	21 2	124 3
	0 21	8 3	17 6	30 5
	1 00	1 0	14 4	0 6
0 12	0 14	14 6	13 0	46 4
	0 21	7 7	11 5	13 1
	1 00	1 0	10 1	0 3

For *carbon dioxide* relations were developed only for the case of no carbon dioxide in inspired air in which case the alveolar ventilation equation is

$$v = \frac{273}{310}\left(\frac{y}{P}\right)\dot{V} \qquad (1\ b)$$

Fick s equation for carbon dioxide can be written

$$\dot{v} = [b - \beta(y)]\,\dot{Q} \qquad (2\ b)$$

An equation of the form

$$\beta(y) = B\left[\frac{y + K}{y + k}\right] \qquad (3\ b)$$

with appropriately chosen constants B K and k can finally be used as an analytical approximation to the observed relation between carbon dioxide concentration and carbon dioxide tension in arterial blood The constants will depend on the carbon dioxide combining capacity of the blood and may be determined so that observed and calculated carbon dioxide concentrations agree for p_aCO_2 = 30 50 and 70 mm Hg A numerical example based on a

carbon dioxide combining power of 48 5 vol per cent at p_aCO_2 = 40 mm Hg is given in table 1b The effect of variations in oxygen tensions on the carbon dioxide combining capacity of the blood (the Haldane effect) is so small that it is disregarded Observed carbon dioxide concentrations at various carbon dioxide tensions from 30 to 70 mm Hg are given in the second column of the table.

Table 1 b

Comparison of carbon dioxide concentrations calculated from (3 b), using the values (4 b) for the constants and carbon dioxide concentrations actually observed at various carbon dioxide tensions, with a carbon dioxide combining power of 0 485 at p_aCO_2 = 40 mm Hg

p_aCO_2 (mm Hg)	C_aCO_2 observ	C_aCO_2 calc
30	0 430	0 430
40	0 485	0 486
50	0 535	0 535
60	0 575	0 575
70	0 610	0 610

The following values of the constants were obtained by fitting (3b) to these observed concentrations

$$B = 1\ 06, \qquad K = 10\ 5, \qquad k = 70 \tag{4 b}$$

Calculated concentrations, obtained from (3b) when the values (4b) are used for the constants, are shown in the last column of the table The agreement between observed and calculated concentrations appears to be perfect for all carbon dioxide tensions from 30 to 70 mm Hg

Elimination of $\beta(y)$ and y from (1b) (2b) and (3b) gives a relation between v, $\dot V$ and $\dot Q$, which is quite similar to the relation (6a) obtained for the oxygen uptake

$$(\dot V - d_1\ v)\ (\dot Q - d_2\ v) = d_0\ v^2 \tag{6 b}$$

where

$$\left.\begin{aligned} d_1 &= 1\ 13\frac{P\,(B-b)}{(bk - BK)} \\ d_2 &= \frac{k}{(bk - BK)} \\ d_0 &= 1\ 13\frac{PB\,(k-K)}{(bk - BK)^2} \end{aligned}\right\} \tag{7 b}$$

In the graphical illustrations of equation (6b) in figure 1b, the barometric pressure was assumed to be 760 mm Hg and no carbon dioxide to be present in inspired air, the values (4b) were used for the constants B, K and k, and the two values 0 50 and 0 60 were selected for venous carbon dioxide concentrations Under these assumptions, the following numerical values were obtained for d_1, d_2 and d_0, and used for the curves in figure 1b

$C_{\bar{v}}CO_2$	d_1	d_2	d_0
0 50	20 2	2 9	95 5
0 60	12 9	2 3	57 2

Summary

The paper contains a theoretical analysis of the relations between alveolar ventilation, pulmonary capillary blood flow, venous gas concentrations and the rates of gas exchanges Assuming an uniform ventilation perfusion ratio and no impairment of diffusion, the analysis is based on the alveolar ventilation equations, Fick's equations and formulas which have been constructed to provide empirical approximations to the observed relations between blood gas tensions and blood gas concentrations

Equations are then derived which relate the rates of gas exchange to alveolar ventilation pulmonary blood flow and venous gas concentrations Graphical presentations of these relations are given in co ordinate systems where alveolar ventilation is plotted along the abscissa and pulmonary blood flow along the ordinate Assuming specified values for venous gas concentrations, points representing constant rates of oxygen uptake will then yield one family of hyperbolas with asymptotes parallel to the co ordinate axes, and points representing equal rates of carbon dioxide output another family In both families the distance between the co ordinate axes and the asymptotes increases with increasing rates of gas exchange as long as composition of inspired air and venous gas concentrations are kept constant Conversely the asymptotes will approach the axes again, if oxygen concentration in inspired air is increased, venous oxygen concentration is reduced, or venous carbon dioxide concentration is increased (see

figures 1a and 1b] Changes in venous gas concentration may, therefore, more or less compensate a reduced capacity to increase either ventilation or blood flow in accordance with metabolic needs

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II

Significance of Moderate Variations in the Ventilation-Blood Flow Ratio for Rates of Gas Exchange and Alveolo-Arterial Gas Gradients

Introduction

It is well known that a variation in the ventilation blood flow ratio throughout the lungs represents a hindrance to efficient gas exchange and an important cause of tension gradients between mixed alveolar air and mixed end pulmonary capillary blood A second source of alveolar capillary tension gradients is impaired diffusion (of importance only for oxygen) while venous admixture may cause an additional gradient between mixed end pulmonary blood and arterial blood In the following analysis of some results of a varying ventilation blood flow ratio it will be assumed that the two last causes of alveolo arterial tension gradients can be disregarded Arterial and mixed end pulmonary blood can then be considered as identical and any alveolo arterial tension gradient can be attributed to variations in $\dot{V}_A/\dot{Q}_c$ alone often referred to as the distribution factor

The significance of this factor as a cause of alveolo arterial tension gradients is easy to demonstrate in numerical examples where given values of ventilation and blood flow are assigned to each of a limited number of lung compartments Farhi and Rahn (4) have also given a numerical evaluation of oxygen gradients based on the assumption of a normal distribution of log ($\dot{V}_A/\dot{Q}_c$) with numerically specified parameters Mean alveolo arterial gradients of 3 4 10 21 and 40 mm Hg were found when the standard deviations of the log ($\dot{V}_A/\dot{Q}_c$) distribution were log (1 25) log (1 5) log (2 0) and log (3 0) respectively the mean value log 0 85 and the oxygen tensions in inspired air and venous blood 150 and 40 mm Hg respectively

All these calculations however are based on specific assumptions regarding the distribution of $\dot{V}_A/\dot{Q}_c$ in the lungs and most of

variables must be continuous and of limited extent, in order that certain analytical approximations shall be permissible

The following main section of the paper will give a general discussion of the relations developed in the Appendix, based on numerical examples, in which the various parameters are given typical values within normal ranges

Numerical Illustrations and discussion of results of the analysis

The main result of the theoretical analysis is that moderate variations in $\dot{V}_A/\dot{Q}_c$ produce mean alveolo capillary gas gradients which under otherwise equal conditions, are approximately proportional to a simple quantitative measure of this variation. This measure which has been denoted by s^2, is, for moderate variations approximately equal to the variance of log $(\dot{V}_A/\dot{Q}_c)$ within the lungs. In symbols, the relations may be expressed as follows

$$p_AO_2 - p_aO_2 = a_1s^2$$

$$p_aCO_2 - p_ACO_2 = b_1s^2$$

$$\frac{\dot{V}O_2\,(\max) - \dot{V}O_2}{\dot{V}O_2\,(\max)} = a_2s^2$$

$$\frac{\dot{V}CO_2\,(\max) - \dot{V}CO_2}{\dot{V}CO_2\,(\max)} = b_2s^2$$

where

p_AO_2 and p_aO_2 are mean alveolar and mean pulmonary capillary oxygen tensions

p_ACO_2 and p_aCO_2 are mean alveolar and mean pulmonary capillary carbon dioxide tensions

$\dot{V}O_2$ and $\dot{V}CO_2$ are the actual rates of gas exchange through lungs with a variation of $\dot{V}_A/\dot{Q}_c$ as indicated by s^2

$\dot{V}O_2$(max) and $\dot{V}CO_2$ (max) are the maximum rates of [illegible]

[illegible] blood flow and venous gas concentrations)

them have been concerned with the effect on oxygen gradients alone Relations of a more general character between variations in $\dot{V}_A/\dot{Q}_c$ and mean alveolo arterial gradients for each of two gases have not been established

It is obvious that variations in $\dot{V}_A/\dot{Q}_c$ in most instances will have a considerably *greater effect on the oxygen gradient than on the* carbon dioxide gradient This is apparent in numerical examples and is also evident from the shapes of the two dissociation curves Carbon dioxide gradients of measurable sizes must, however, be taken into consideration as possible results of uneven $\dot{V}_A/\dot{Q}_c$ A significantly increased physiological dead space commonly accepted as a standard criterion for unequal ventilation blood flow ratios, is actually equivalent to a carbon dioxide gradient between arterial blood and mixed alveolar air A theoretical relation between CO_2 gradient and increased physiological dead space can easily be established, as will be shown later

The ideal objective of a theoretical analysis would be to express both gradients, and the rates of gas exchange which can be attained under specified conditions, as functions of some general measure of the variation of $\dot{V}_A/\dot{Q}_c$ throughout the lungs Even if the numerical value of this measure were unknown in all actual cases the general relations would permit a study of the significance of various parameters and a comparison of the effects of ventilation blood flow disturbances on the two gas gradients under various conditions

A general analytical solution of the problem applicable to all conceivable physiological and pathological conditions is probably impossible to find because of the great number of variables and the difficulties in expressing some of them in a quantitative way An approximate solution, applicable under certain conditions and within a limited range of variation is given in the Appendix to the present paper The analysis is based on a simple model of the lungs and pulmonary circulation and on theoretical definitions

of gas exchange which can be attained are then related to the variance of $\dot{V}_A/\dot{Q}_c$ throughout the lungs No particular assumptions are made regarding *the type of distribution of* $\dot{V}_A/\dot{Q}_c$ in the lungs except that the variation of this ratio, and of certain other

flow ratio leads to a larger carbon dioxide gradient, and to a smaller oxygen gradient, if venous oxygen concentration is 0 15 When venous oxygen concentration is 0 12, the highest oxygen gradient is obtained at an intermediate overall ventilation blood flow ratio of 0 8 This means that the significance of carbon dioxide gradients, originating from uneven $\dot{V}_A/\dot{Q}_c$, increases with increasing predominance of hyperventilated areas in relation to hypo ventilated areas within the lungs Increasing carbon dioxide gradients are, in turn, equivalent to increasing physiological dead spaces, as will be discussed later

The gas gradients between mean alveolar air and mean pul monary capillary blood, produced by a varying $\dot{V}_A/\dot{Q}_c$ can be illustrated graphically in Rahn Fenn diagrams (figure 1) Let the

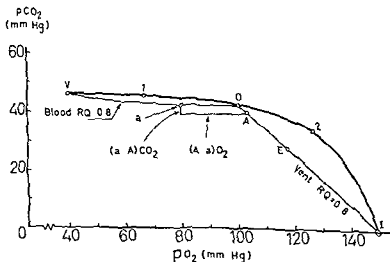


Figure 1

Schematic illustration in Rahn Fenn diagram of arterio alveolo oxygen and carbon dioxide gradients caused by uneven distribution of ventilation and blood flow

points I and V denote gas tensions in inspired air and ...

a_1 b_1, a_2 and b_2 are constants which depend on venous gas concentrations gas combining capacities *of arterial blood, composition* of inspired air and on the ratio between total alveolar ventilation and total pulmonary blood flow, but which are independent of the variations of $\dot{V}_A/\dot{Q}_c$ within the lungs

s^2 is a quantitative measure of the variations of $\dot{V}_A/\dot{Q}_c$ within the lungs

Tables 1a and 1b give numerical examples of oxygen and carbon dioxide gradients expressed by the variance s^2 under various specified conditions For each gas results are given for two venous

Tables 1 a—b

Mean alveolo capillary gas gradients caused by uneven distribution of *alveolar ventilation and pulmonary blood flow*

$P_B = 760$ mm Hg $F_IO_2 = 0\ 21$ $F_ICO_2 = 0$

s^2 — measure of variation of $\dot{V}_A/\dot{Q}_c$

a Oxygen gradients

Total $\dot{V}_A$ / Total $\dot{Q}_c$	Venous O_2 conc	
	0 15	0 12
0 5	$62s^2$	$63s^2$
0 8	$54s^2$	$80s^2$
1 2	$38s^2$	$66s^2$

b Carbon dioxide gradients

Total $\dot{V}_A$ / Total $\dot{Q}_c$	Venous CO_2 conc	
	0 50	0 60
0 5	$3\ 6s^2$	$7\ 1s^2$
0 8	$4\ 9s^2$	$9\ 1s^2$
1 2	$6\ 1s^2$	10 [illegible]s^2

gas concentrations and three ratios between total alveolar ventilation and total pulmonary blood flow Ordinary atmospheric air at a barometric pressure of 760 mm Hg is assumed to be inspired and approximately normal values stated in the Appendix are chosen *for gas combining capacities of arterial blood*

Very roughly the oxygen gradients have an order of magnitude about 10 times greater than the carbon dioxide gradients — a result which was to be expected because of the different shapes of the two dissociation curves Both gradients depend however *on the venous gas concentrations* and on the ratio between total alveolar ventilation and total pulmonary blood flow The oxygen gradient increases with decreasing venous oxygen concentration and the carbon dioxide gradient increases *with increasing venous* carbon dioxide concentration Increasing overall ventilation blood

flow ratio leads to a larger carbon dioxide gradient and to a smaller oxygen gradient, if venous oxygen concentration is 0 15 When venous oxygen concentration is 0 12 the highest oxygen gradient is obtained at an intermediate overall ventilation blood flow ratio of 0 8 This means that the significance of carbon dioxide gradients originating from uneven $\dot{V}_A/\dot{Q}_c$ increases with increasing predominance of hyperventilated areas in relation to hypoventilated areas within the lungs Increasing carbon dioxide gradients are, in turn, equivalent to increasing physiological dead spaces as will be discussed later

The gas gradients between mean alveolar air and mean pulmonary capillary blood, produced by a varying $\dot{V}_A/\dot{Q}_c$ can be illustrated graphically in Rahn Fenn diagrams (figure 1) Let the

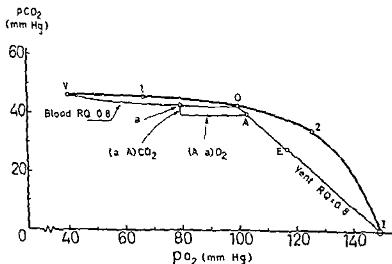


Figure 1
Schematic illustration in Rahn Fenn diagram of arterio alveolo oxygen and carbon dioxide gradients caused by uneven distribution of ventilation and blood flow

points I and V denote *gas tensions in inspired air* and in mixed venous blood and the heavy curve between these two points the tensions at which gas can exchange between alveoli and capillary blood Let us further assume a respiratory quotient of 0 8 *for the*

total gas exchange and a mean $\dot{V}_A/\dot{Q}_c$ of about 0 8 The point 0 on the heavy curve would then represent the alveolar (and end capillary) gas tensions which would be found with a constant $\dot{V}_A/\dot{Q}_c$ = 0 8 *throughout the lungs It is the point where the* ventilation $R\ Q$ = 0 8 line and the blood $R\ Q$ = 0 8 curve, both shown in the figure, intersect

If $\dot{V}_A/\dot{Q}_c$ varies, gas tension of the individual alveoli will be distributed along the heavy curve around the point 0 Let us from a practical point of view, assume that the range of variation is from point 1 to point 2 The first of these points corresponds to a $\dot{V}_A/\dot{Q}_c$ of approximately 0 3 and a $R\ Q$ of approximately 0 5, the second to a $\dot{V}_A/\dot{Q}_c$ *of about 2 0 and a* $R\ Q$ *of about 1 6*

The points representing mixed expired alveolar air and mixed end capillary pulmonary blood will not, however, be on the heavy curve in cases with a varying $\dot{V}_A/\dot{Q}_c$ If the $R\ Q$ for the total gas exchange is 0 8, the point indicating mean alveolar gas tension (A) must be somewhere on the ventilation $R\ Q$ = 0 8 line, below point 0, but above the level of point 2 Similarly, the point representing arterial blood (a), which under the present assumptions *will be identical with mixed end capillary blood, must* be somewhere on the blood $R\ Q$ = 0 8 curve, to the left of point 0 but to the right of point 1 The horizontal distance between the points "a" and A is the mean alveolo capillary oxygen gradient, while the vertical distance between the same points is the mean capillary alveolo carbon dioxide gradient

A horizontal line through the point "a' will intersect the ventilation $R\ Q$ = 0 8 line slightly above point 0 (not shown on the figure) This point represents *the gas tensions in the so called* "ideal' alveolar air (Riley (8)), i e a theoretically conceived gas mixture with the same carbon dioxide tension as the arterial blood, and with an oxygen tension computed from the alveolar air equation — using the $R\ Q$ for the total gas exchange through the lungs (0 8 in the present illustration) It is seen that in cases with uneven $\dot{V}_A/\dot{Q}_c$, the oxygen tension in ' ideal alveolar air will be smaller, and the carbon dioxide tensions greater than the corresponding *tensions in the theoretically conceived mean* (expired) alveolar air, as represented by the point A For practical purposes, the differences will, however, usually be negligible because of the ordinarily small arterio *alveolo carbon dioxide gradient*

It should be emphasized that in practice, point A is difficult to define and to determine, because no satisfactory method is available to get representative samples of mixed expired alveolar air Nevertheless, an analysis of theoretical relations, based on a simple and well defined model of the lungs, may be useful for the interpretation of actually observed measures in pulmonary physiology

It may be of interest to compare oxygen gradients obtained from the present analysis with those computed by Farhi and Rahn (4) for similar conditions Their computations were based on a normal distribution of log ($\dot{V}_A/\dot{Q}_c$) around a mean of log (0 85), with standard deviations equal to log (1 25), log (1 50), log (2 00) and log (3 00) a venous oxygen tension of 40 mm Hg, and a venous carbon dioxide tension of 46 mm Hg The last column of table 2a shows the oxygen gradients obtained under these conditions when atmospheric air was inspired

For relatively small values, the quantity s^2 is approximately equal to the standard deviation of $\log_e (\dot{V}_A/\dot{Q}_c)$, as shown in the Appendix Estimates of s^2 obtained with this approximation are given in second column of table 2a for the four standard deviations chosen by Farhi and Rahn

The conditions included in table 1a which are most similar to those used by Farhi and Rahn, are $C_vO_2 = 0$ 15 and an over all $\dot{V}_A/\dot{Q}_c = 0$ 8 To compute the gradients, the values for s^2 in the

Table 2 a

Comparison of mean alveolo-capillary oxygen gradients caused by a varying $\dot{V}_A/\dot{Q}_c$ computed by Farhi and Rahn, and estimated from the present analysis

$P_B = 760$ mm Hg $F_IO_2 = 0\ 21$, $C_vO_2 = 0\ 15$

Overall $\dot{V}_A/\dot{Q}_c = 0\ 8$ s^2 = variance of $\log_e(\dot{V}_A/\dot{Q}_c)$

Stand dev of log $\left(\frac{\dot{V}_A}{\dot{Q}_c}\right)$	s^2	O_2 grad from $[p_AO_2 - p_aO_2] = 54s^2$	O_2 gradient computed by Farhi and Rahn
log 1 25	0 05	2 7	3 4
log 1 50	0 16	8 6	10
log 2 00	0 48	25 9	21
log 3 00	1 21	65 3	40

total gas exchange and a mean $\dot{V}_A/\dot{Q}_c$ of about 0 8 The point 0 on the heavy curve would then represent the alveolar (and end capillary) gas tensions which would be *found with a constant* $\dot{V}_A/\dot{Q}_c = 0\ 8$ throughout the lungs It is the point where the ventilation $R\ Q = 0\ 8$ line and the blood $R\ Q = 0\ 8$ curve, both *shown in the figure intersect*

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A horizontal line through the point "a" will intersect the ventilation $R\ Q = 0\ 8$ line slightly above point 0 (not shown on the figure) This point represents the gas tensions in the so called "ideal" alveolar air (*Riley (8)*) *i e a theoretically conceived gas* mixture with the same carbon dioxide tension as the arterial blood and with an oxygen tension computed from the alveolar air equation — using *the $R\ Q$ for the total gas exchange through the* lungs (0 8 in the present illustration) It is seen that in cases with uneven $\dot{V}_A/\dot{Q}_c$ the oxygen tension in "ideal" alveolar air will be smaller, and the carbon dioxide tensions greater *than the corre*sponding tensions in the theoretically conceived mean (expired) alveolar air, as represented by the point A For practical purposes the differences will, however, usually be *negligible because of the* ordinarily small arterio alveolo carbon dioxide gradient

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3

second column of table 2a should then be inserted in $64s^2$ (second figure in left-hand column of table 1a). The results, presented in the *third column of table 2a,* agree reasonably well with those computed by Farhi and Rahn, except the result obtained with the largest value of s^2. However, the approximation used in the estimation of s^2, as well as other approximations used in the present analysis, become very rough for such large variations of the ventilation-blood flow ratio.

The existence of carbon dioxide gradients between arterial blood and mixed expired alveolar air is theoretically equivalent to a difference between physiological and anatomical dead-space. The *anatomical dead-space is related to carbon dioxide tensions* in alveolar air and in expired air by the Bohr equation:

$$V_{D(An)} = \frac{p_A CO_2 - p_E CO_2}{p_A CO_2} V_T$$

where V_T is tidal volume.

The physiological dead-space is obtained by substituting $p_a CO_2$ for $p_A CO_2$ in this formula:

$$V_{D(Ph)} = \frac{p_a CO_2 - p_E CO_2}{p_a CO_2} V_T$$

Subtraction of the first equation from the last gives.

$$V_{D(Ph)} - V_{D(An)} = \left[\frac{p_E CO_2 \times V_T}{p_A CO_2}\right] \left[\frac{p_a CO_2 - p_A CO_2}{p_a CO_2}\right]$$

The quantity in the first bracket on the right side of this equation is the difference between tidal volume and anatomical dead-space, and *this difference is usually about 320 ml. For* $p_a CO_2$ we may, without too large errors, use the value 40 mm Hg, even if its value may be considerably greater under pathological conditions. An impression of the magnitude of the difference between physiological and anatomical dead-space, for given carbon dioxide gradients, may then be obtained from:

$$V_{D(Ph)} - V_{D(An)} \approx 8\,(p_a CO_2 - p_A CO_2)$$

with volumes measured in ml and tensions in mm *Hg*. Considering that the oxygen gradients produced by an uneven $\dot{V}_A/\dot{Q}_c$, according to tables 1a-b, are roughly ten times greater than the carbon

Tables 3 a—b

Mean alveolo-capillary oxygen gradients and differences between physiological and anatomical dead space caused by variations of $\dot{V}_A/\dot{Q}_c$ with a variance $s^2 = 0.48$

$P_B = 760$ mm Hg, $F_IO_2 = 0.21$, $F_ICO_2 = 0$

a Oxygen gradients

Total $\dot{V}_A$ / Total $\dot{Q}_c$	Venous O_2 conc 0.15	Venous O_2 conc 0.12
0.5	30 mm	30 mm
0.8	26 ,,	28 ,,
1.2	18 ,,	32 ,,

b Difference phys anat dead space

Total $\dot{V}_A$ / Total $\dot{Q}_c$	Venous CO_2 conc 0.50	Venous CO_2 conc 0.60
0.5	14 ml	27 ml
0.8	19 ,,	35 ,,
1.2	24 ,,	41 ,,

dioxide gradients, one would expect that the oxygen gradient (measured in mm Hg) and the difference between physiological and anatomical dead space (measured in ml) would have approximately the same order of magnitude — provided that both are caused exclusively by moderate variations in $\dot{V}_A/\dot{Q}_c$. This relation will however, depend on several factors, particularly on venous gas concentrations and on the over all ventilation blood flow ratio

Tables 3a and 3b show oxygen gradients and differences between physiological and anatomical dead spaces, computed from the data in tables 1a and 1b, when $s^2 = 0.48$. A comparison of these calculated values with actually observed values will be uncertain, partly because of relatively great experimental errors in the determination of physiological dead space (*Aksnes (1)*), and partly because of uncertain information on the size of anatomical dead-space. Observations indicate, however, that differences between physiological and anatomical dead space which are clearly greater than those shown *in table 3b*, can occur together with oxygen gradients of the magnitude indicated in table 3a (Aksnes (2)). A possible reason for this is that venous carbon dioxide content in such cases frequently is considerably higher than has been assumed in the present calculations. Moreover, the trend in table 3b suggests that a combination of a large physiological dead space with a relatively low oxygen gradient could be expected when there is a relative predominance of hyperventilated areas in the lungs, while the reverse combination should occur when there

is a predominance of hypoventilation (or impairment of dif fusion)

Tables 4a and 4b show reductions in rates of gas exchange as fractions of the maximum rates which the same total alveolar ventilation and the same total pulmonary blood flow could give if $\dot{V}_A/\dot{Q}_c$ had been constant and conditions otherwise unchanged It is seen that uneven distribution of ventilation and blood flow is generally a greater hindrance for oxygen uptake than for carbon dioxide output Its significance for carbon dioxide output in creases however with increasing degree of relative hyperventila tion in the lungs If $s^2 = 0.48$ (a value which appears reasonable according to the oxygen gradients given in table 2a) and the overall $\dot{V}_A/\dot{Q}_c = 0.8$ the reduction in the efficiency of oxygen up take will be about 11 17 per cent and the reduction in efficiency of carbon dioxide output about 6 7 per cent

Finally table 5a shows that the inhibitory effect of a non uniform $\dot{V}_A/\dot{Q}_c$ on the oxygen uptake practically disappears when pure oxygen is inspired It is reasonable to assume that inspiration of pure oxygen will then also eliminate the oxygen gradient more or less This however could not be verified by direct calculations because the formulas developed to compute oxygen gradient be come very inaccurate when pure oxygen is inspired

APPENDIX

Definitions of Symbols and Basic Concepts used in the Analysis

To avoid a heavy overloading of formulas and equations the con ventional standard symbols (Federation Proceedings 9 602—605) will be replaced by shorter denotations which have been defined in terms of the standard symbols in the Appendix of the preceding paper (page 15)

The following model of the lungs and pulmonary circulation will be used as a basis for definitions and analysis of the problem The total lung parenchyma which contributes to the gas exchange is considered as being composed of a great number of very small *elements* These elements may be thought to be numbered in a certain sequence according to a system which can be chosen

Tables 4 a—b

Relative reductions in rates of gas exchange caused by uneven distribution of alveolar ventilation and pulmonary blood flow under specified conditions

$P_B = 760$ mm Hg $F_IO_2 = 0.21$, $F_ICO_2 = 0$

s^2 — measure of variation of $\dot{V}_A/\dot{Q}_c$

a Reduction in O_2 uptake

Total $\dot{V}_A$ / Total $\dot{Q}_c$	Venous O_2 conc	
	0.15	0.12
0.5	$0.37s^2$	$0.39s^2$
0.8	$0.23s^2$	$0.35s^2$
1.2	$0.13s^2$	$0.20s^2$

b Reduction in CO_2-output

Total $\dot{V}_A$ / Total $\dot{Q}_c$	Venous CO_2 conc	
	0.50	0.60
0.5	$0.09s^2$	$0.11s^2$
0.8	$0.12s^2$	$0.14s^2$
1.2	$0.14s^2$	$0.16s^2$

Table 5 a

Relative reductions in rate of oxygen uptake caused by uneven distribution of alveolar ventilation and pulmonary blood flow when pure oxygen is inspired and conditions otherwise as specified

$P_B = 760$ mm Hg s^2 — measure of variation of $\dot{V}_A/\dot{Q}_c$

Total $\dot{V}_A$ / Total $\dot{Q}_c$	Venous O_2 conc	
	0.15	0.12
0.5	$0.01s^2$	$0.01s^2$
0.8	$0.01s^2$	$0.01s^2$
1.2	$0.004s^2$	$0.004s^2$

arbitrarily so that each element can be identified by the value of a single variable which will be denoted by L If the elements are very small L may be regarded as a continuous variable, and the size of an element at location L as an infinitesimal increment dL of the variable The unit of L can be chosen arbitrarily and it will be convenient for the subsequent analysis to define it so that

$$\int dL = 1$$

Th...

assı

trit ... gas exchange

Alveolar ventilation and pulmonary capillary blood are assumed to be uniform within each element The value of these two variables

for a given element can then be considered as proportional to its size and to a function of its location, i e

$$d\dot{V} = \dot{V}(L)\,dL, \qquad d\dot{Q} = \dot{Q}(L)\,dL$$

where $\dot{V}(L)$ and $\dot{Q}(L)$ can be interpreted as ventilation and blood flow per "unit of lung tissue" (as measured by the variable L) at the site of the element

Oxygen uptake and carbon dioxide output in the element can be expressed similarly as

$$du = u(L)\,dL, \qquad dv = v(L)\,dL$$

The symbols $\dot{V}$, $\dot{Q}$ etc, may be used instead of $\dot{V}(L)$, $\dot{Q}(L)$ etc, with the understanding thay they are always considered as functions of L (and not as total alveolar ventilation etc)

The total ventilation, blood flow, O_2 uptake and CO_2 output will be equal to the average values of these variables per "unit of lung tissue" and will be denoted by

$$\dot{V}_T = \int \dot{V}(L)\,dL, \qquad \dot{Q}_T = \int \dot{Q}(L)\,dL$$

$$u_T = \int u(L)\,dL, \qquad v_T = \int v(L)\,dL$$

The alveolar oxygen and carbon dioxide tension in a lung element, $x = x(L)$ and $y = y(L)$ are, of course, also functions of the variable L, but independent of the size of the element, dL Because no diffusion gradients are assumed to exist, the same gas tensions will be found in the pulmonary capillary blood which comes from the element

The gas tensions which would be found in mixed expired alveolar air, without admixture of air from the anatomical dead space will be referred to as *mean alveolar gas tensions* and defined by

$$x_A = \frac{1}{\dot{V}_T}\int \dot{V}(L)\,x(L)\,dL \tag{1a}$$

$$y_A = \frac{1}{\dot{V}_T}\int \dot{V}(L)\,y(L)\,dL \tag{1b}$$

It can be shown that x_A and y_A defined in this way satisfy the alveolar air equation, regardless of variations in $\dot{V}/\dot{Q}$, $x(L)$ and $y(L)$

The gas tensions which would be found in mixed pulmonary end capillary blood (or in arterial blood under the assumptions made here) will be referred to as *mean capillary gas tensions*, and denoted by x_a and y_a. They are defined by the equations

$$\alpha(x_a) = \frac{1}{\dot{Q}_T} \int \dot{Q}(L)\, \alpha[x(L)]\, dL \tag{2a}$$

$$\beta(y_a) = \frac{1}{\dot{Q}_T} \int \dot{Q}(L)\, \beta[y(L)]\, dL \tag{2 b}$$

The differences $(x_A - x_a)$ and $(y_a - y_A)$ will be called the mean alveolo capillary oxygen gradient and the mean capillary alveolo carbon dioxide gradient respectively. Under the assumptions which have been made, both mean differences will be zero if $\dot{V}/\dot{Q}$ is constant throughout the lungs, but may become positive if $\dot{V}/\dot{Q}$ varies, even if the gradients within the individual lung elements are all zero. The main objective of the following analysis is to develop an approximate analytical relation between the degree of variation of $\dot{V}/\dot{Q}$ and each of the two mean gradients. As a by-product of this analysis, we will also get relations between rates of gas exchange through lungs with uneven distribution of ventilation and perfusion, and the maximum rates which could be obtained without variation in $\dot{V}/\dot{Q}$ under otherwise indentical conditions.

Analysis of relations between rates of gas exchange, mean alveolo capillary gas gradients and variations in the ventilation blood flow ratio

Analytical expressions for the mean alveolo capillary gas gradients will be derived from *(1a)* and *(2a)* for oxygen, and from *(1b)* and *(2b)* for carbon dioxide. In addition, it is necessary to have analytical approximations to experimentally observed relations between gas tensions and gas concentrations in arterial blood. It was shown in the preceding paper (pages 16 and 20) that equations of the form

$$\alpha(x) = A\frac{(x - H)}{(x - h)} \tag{3 a}$$

$$\beta(y) = B\frac{(y + K)}{(y + k)} \tag{3 b}$$

for a given element can then be considered as proportional to its size and to a function of its location, i e

$$d\dot{V} = \dot{V}(L)\,dL, \qquad d\dot{Q} = \dot{Q}(L)\,dL$$

where $\dot{V}(L)$ and $\dot{Q}(L)$ can be interpreted as ventilation and blood flow per "unit of lung tissue" (as measured by the variable L) at the site of the element

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The symbols $\dot{V}$, $\dot{Q}$ etc, may be used instead of $\dot{V}(L)$, $\dot{Q}(L)$ etc, with the understanding thay they are always considered as functions of L (and not as total alveolar ventilation etc)

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$$\alpha(x_a) = \frac{1}{Q_T}\int Q(L)\,\alpha[x(L)]\,dL \tag{2a}$$

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$$\alpha(x_a) = \frac{1}{\dot{Q}_T}\int \dot{Q}(L)\,\alpha[x(L)]\,dL \tag{2a}$$

$$\beta(y_a) = \frac{1}{\dot{Q}_T}\int \dot{Q}(L)\,\beta[y(L)]\,dL \tag{2b}$$

The differences $(x_A - x_a)$ and $(y_a - y_A)$ will be called the mean alveolo capillary oxygen gradient and the mean capillary alveolo carbon dioxide gradient respectively. Under the assumptions which have been made, both mean differences will be zero if $\dot{V}/\dot{Q}$ is constant throughout the lungs, but may become positive if $\dot{V}/\dot{Q}$ varies, even if the gradients within the individual lung elements are all zero. The main objective of the following analysis is to develop an approximate analytical relation between the degree of variation of $\dot{V}/\dot{Q}$ and each of the two mean gradients. As a by-product of this analysis, we will also get relations between rates of gas exchange through lungs with uneven distribution of ventilation and perfusion, and the maximum rates which could be obtained without variation in $\dot{V}/\dot{Q}$ under otherwise indentical conditions.

Analysis of relations between rates of gas exchange, mean alveolo capillary gas gradients and variations in the ventilation-blood flow ratio

Analytical expressions for the mean alveolo capillary gas gradients will be derived from *(1a)* and *(2a)* for oxygen, and from *(1b)* and *(2b)* for carbon dioxide. In addition, it is necessary to have analytical approximations to experimentally observed relations between gas tensions and gas concentrations in arterial blood. It was shown in the preceding paper (pages 16 and 20) that equations of the form

$$\alpha(x) = A\,\frac{(x - H)}{(x - h)} \tag{3a}$$

$$\beta(y) = B\,\frac{(y + K)}{(y + k)} \tag{3b}$$

for a given element can then be considered as proportional to its size and to a function of its location, i e

$$d\dot{V} = \dot{V}(L)\,dL, \qquad d\dot{Q} = \dot{Q}(L)\,dL$$

where $\dot{V}(L)$ and $\dot{Q}(L)$ can be interpreted as ventilation and blood flow per "unit of lung tissue" (as measured by the variable L) at the site of the element

Oxygen uptake and carbon dioxide output in the element can be expressed similarly as

$$du = u(L)\,dL, \qquad dv = v(L)\,dL$$

The symbols $\dot{V}$, $\dot{Q}$ etc, may be used instead of $\dot{V}(L)$, $\dot{Q}(L)$ etc, with the understanding thay they are always considered as functions of L (and not as total alveolar ventilation etc)

The total ventilation, blood flow, O_2 uptake and CO_2 output will be equal to the average values of these variables per "unit of lung tissue" and will be denoted by

$$\dot{V}_T = \int \dot{V}(L)\,dL, \qquad \dot{Q}_T = \int \dot{Q}(L)\,dL$$

$$u_T = \int u(L)\,dL, \qquad v_T = \int v(L)\,dL$$

The alveolar oxygen and carbon dioxide tension in a lung element, $x = x(L)$ and $y = y(L)$ are, of course, also functions of the variable L, but independent of the size of the element, dL Because no diffusion gradients are assumed to exist, the same gas tensions will be found in the pulmonary capillary blood which comes from the element

The gas tensions which would be found in mixed expired alveolar air, without admixture of air from the anatomical dead space, will be referred to as *mean alveolar gas tensions* and defined by

$$x_A = \frac{1}{\dot{V}_T}\int \dot{V}(L)\,x(L)\,dL \tag{1a}$$

$$y_A = \frac{1}{\dot{V}_T}\int \dot{V}(L)\,y(L)\,dL \tag{1b}$$

It can be shown that x_A and y_A defined in this way *satisfy the* alveolar air equation, regardless of variations in $\dot{V}/\dot{Q}$, $x(L)$ and $y(L)$

The gas tensions which would be found in mixed pulmonary end capillary blood (or in arterial blood under the assumptions made here) will be referred to as *mean capillary gas tensions* and denoted by x_a and y_a. They are defined by the equations

$$\alpha(x_a) = \frac{1}{\dot{Q}_T} \int \dot{Q}(L)\, \alpha[x(L)]\, dL \tag{2a}$$

$$\beta(y_a) = \frac{1}{\dot{Q}_T} \int \dot{Q}(L)\, \beta[y(L)]\, dL \tag{2 b}$$

The differences $(x_A - x_a)$ and $(y_a - y_A)$ will be called the mean alveolo capillary oxygen gradient and the mean capillary alveolo carbon dioxide gradient respectively. Under the assumptions which have been made both mean differences will be zero if $\dot{V}/\dot{Q}$ is constant throughout the lungs but may become positive if $\dot{V}/\dot{Q}$ varies even if the gradients within the individual lung elements are all zero. The main objective of the following analysis is to develop an approximate analytical relation between the degree of variation of $\dot{V}/\dot{Q}$ and each of the two mean gradients. As a by product of this analysis we will also get relations between rates of gas exchange through lungs with uneven distribution of ventilation and perfusion and the maximum rates which could be obtained without variation in $\dot{V}/\dot{Q}$ under otherwise indentical conditions.

Analysis of relations between rates of gas exchange mean alveolo capillary gas gradients and variations in the ventilation blood flow ratio

Analytical expressions for the mean alveolo capillary gas gradients will be derived from *(1a)* and *(2a)* for oxygen and from *(1b)* and *(2b)* for carbon dioxide. In addition it is necessary to have analytical approximations to experimentally observed relations between gas tensions and gas concentrations in arterial blood. It was shown in the preceding paper (pages 16 and 20) that equations of the form

$$\alpha(x) = A\frac{(x - H)}{(x - h)} \tag{3 a}$$

$$\beta(y) = B\frac{(y + K)}{(y + k)} \tag{3 b}$$

with the denotations given in the preceding paper (page 15), and with appropriately chosen constants A, H, h, and B, K, k, may give good approximations to these relations, if the range of variation is not too wide. The constants will depend on gas combining capacities of the blood and may be determined by fitting (3a) or (3b) to three appropriately chosen points of the observed relations.

Because both gas tensions and the respiratory quotient will vary from place to place in lungs with a varying ventilation blood flow ratio Bohr effect and Haldane effect are difficult to take into consideration in the relations. The Bohr effect is generally the more important, but local variations in p_ACO_2 within the lungs are, on the other hand, ordinarily small for a given $p_{\bar{V}}CO_2$. Local variations in p_AO_2 may be considerably greater; their influence on the CO_2 combining capacity of the blood, however, is relatively small, because the Haldane effect is a less significant factor than the Bohr effect. For a given subject with a specified $p_{\bar{V}}CO_2$ it should be permissible, therefore, to use (3a) and (3b) with fixed values for the constants, as first approximations, without taking Bohr effect or Haldane effect into consideration. It should be noted, however, that different values for the constants in (3a) may be necessary in subjects with great differences in $p_{\bar{V}}CO_2$. As shown in table 2a of the preceding paper (page 18), fair approximations can be obtained with

$$A = 0.222 \qquad H = 15 \qquad \text{and } h = 4 \tag{4 a}$$

when oxygen tension is above 60 mm Hg, carbon dioxide tension between 40 and 60 mm Hg and the hemoglobin content 15 g/100 ml blood. These values, and the values

$$B = 1.06 \qquad K = 10.5 \qquad k = 70 \tag{4 b}$$

based on a carbon dioxide combining power of 48.5 vol. per cent at $p_aCO_2 = 40$ mm Hg and used in table 1b of the preceding paper (page 00), will be chosen in the numerical illustrations of the results of the analysis.

Expressions for the mean capillary gas tensions, x_a and y_a, will then be derived from (2a—b), (3a—b) and Fick's equations. For a single lung element, these equations can be written

$$udL = [a(x) - a]\dot{Q}dL \tag{5 a}$$

$$vdL = [b - \beta(y)]\dot{Q}dL \tag{5 b}$$

Integration of ($5a$) over all lung elements, together with ($2a$), gives

$$u_T = [a(x_a) - a]\dot{Q}_T$$

Inserting ($3a$) for $a(x_a)$ and solving for x_a we get

$$x_a = h + \frac{A(H-h)\dot{Q}_T}{(A-a)\dot{Q}_T - u_T} \tag{6 a}$$

The corresponding expression for y_a derived in a similar way is

$$y_a = -k + \frac{B(k-K)\dot{Q}_T}{(B-b)\dot{Q}_T + v_T} \tag{6 b}$$

An expression for x_A is obtained by inserting ($3a$) for $a(x)$ in ($5a$), solving for x and inserting the solution in the integrand of ($1a$)

$$x_A = h + \frac{A(H-h)}{\dot{V}_T}\int \frac{\dot{V}\dot{Q}}{[(A-a)\dot{Q} - u]}dL \tag{7 a}$$

In this expression, the value of u for any lung element will be a function of $\dot{V}$ and $\dot{Q}$ for the same element, because oxygen con centration in mixed venous blood, composition of inspired air, and hemoglobin content of the blood are the same for all elements As shown in the preceding paper (page 17), the relation between u $\dot{V}$ and $\dot{Q}$ can be approximately described by an equation of the form

$$(\dot{V} - c_1u)(\dot{Q} - c_2u) = c_0u^2 \tag{8 a}$$

with the constants c_1 c_2 and c_0 defined by ($7a$) page 17 of the preceding paper An equation of the same form was found for carbon dioxide (equations ($6b$—$7b$) page 21)

The following abbreviated denotations will be introduced to simplify subsequent formulas The values which the variable $u = u(\dot{V}, \dot{Q})$ and its partial derivatives of first and second order assume, when $\dot{V} = \dot{V}_T$ and $\dot{Q} = \dot{Q}_T$ will be denoted as follows

$$u_0 = u(\dot{V}_T\ \dot{Q}_T) \qquad u_1 = \frac{\delta u(\dot{V}_T\ \dot{Q}_T)}{\delta \dot{V}} \qquad u_2 = \frac{\delta u(\dot{V}_T\ \dot{Q}_T)}{\delta \dot{Q}}$$

$$u_{11} = \frac{\delta^2 u(\dot{V}_T\ \dot{Q}_T)}{\delta \dot{V}^2}, \qquad u_{22} = \frac{\delta^2 u(\dot{V}_T\ \dot{Q}_T)}{\delta \dot{Q}^2} \qquad u_{12} = \frac{\delta^2 u(\dot{V}_T, \dot{Q}_T)}{\delta \dot{V}\,\delta \dot{Q}}$$

The integrand in (7a), considered as a function of $\dot{V}$ and $\dot{Q}$, will be denoted by

$$F(\dot{V}, \dot{Q}) = \frac{\dot{V}\dot{Q}}{(A-a)\dot{Q} - u(\dot{V}, \dot{Q})} \tag{10 a}$$

The symbols

$$S_1^2 = \int (\dot{V} - \dot{V}_T)^2 dL, \qquad S_2^2 = \int (\dot{Q} - \dot{Q}_T)^2 dL$$
$$r = \frac{1}{S_1 S_2} \int (\dot{V} - \dot{V}_T)(\dot{Q} - \dot{Q}_T) dL \tag{11}$$

will be used for quantities which can be interpreted as variances and correlation coefficient in the joint distribution of all lung elements by the values of the variables $\dot{V}$ and $\dot{Q}$

A variable t will be defined as follows

$$t = \left[\frac{\dot{V}}{\dot{V}_T} - \frac{\dot{Q}}{\dot{Q}_T}\right] \tag{12}$$

Its variance will be denoted by s^2, and be equal to

$$s^2 = \int t^2 dL = \frac{S_1^2}{\dot{V}_T^2} + \frac{S_2^2}{\dot{Q}_T^2} - 2\frac{S_1 S_2}{\dot{V}_T \dot{Q}_T} r \tag{13}$$

The quantity s^2 can be taken as a measure of the degree to which $\dot{V}$ and $\dot{Q}$ vary independently of each other in the lungs If we have

$$\frac{\dot{V}}{\dot{Q}} = \frac{\dot{V}_T}{\dot{Q}_T}$$

for all values of L, t will obviously be $= O$ for all elements, and $s^2 = O$ For moderate variation of $\dot{V}$ and $\dot{Q}$ in relation to $\dot{V}_T$ and $\dot{Q}_T$ we may write approximately

$$\frac{\dot{V}}{\dot{V}_T} \approx \left(1 + \ln\frac{\dot{V}}{\dot{V}_T}\right) \qquad \frac{\dot{Q}}{\dot{Q}_T} \approx \left(1 + \ln\frac{\dot{Q}}{\dot{Q}_T}\right)$$

which gives

$$t \approx \ln\left(\frac{\dot{V}}{\dot{V}_T}\right) - \ln\left(\frac{\dot{Q}}{\dot{Q}_T}\right) = \ln\left(\frac{\dot{V}}{\dot{Q}}\right) - \ln\left(\frac{\dot{V}_T}{\dot{Q}_T}\right) \tag{14}$$

For small variations of the ventilation blood flow ratio, the variance s^2 of the variable t will, therefore, be approximately equal to the variance of the logarithm of the ventilation blood flow ratio throughout the lungs

To proceed in the evaluation of the difference $(x_A - x_a)$, it is then necessary to find approximate expressions for the two integrals

$$u_T = \int u(\dot{V}, \dot{Q})\, dL \quad \text{and} \quad \int F(\dot{V}, \dot{Q})\, dL$$

which appear in (6a) and (7a) For this purpose, the two integrands are expanded in Taylor series by the variables $\dot{V}$ and $\dot{Q}$ and terms of more than second order are then disregarded Using the denotations indicated by (9a) and (11), and observing that

$$\int (\dot{V} - \dot{V}_T)\, dL = 0, \qquad \int (\dot{Q} - \dot{Q}_T)\, dL = 0$$

we obtain for the first of the two integrals

$$u_T \approx u_0 + \frac{1}{2} u_{11} S_1^2 + \frac{1}{2} u_{22} S_2^2 + u_{12} S_1 S_2 r \qquad (15\ a)$$

This expression can be simplified, when we consider that the relation $u = u(\dot{V}, \dot{Q})$ must be homogeneous in u, $\dot{V}$ and $\dot{Q}$ (all three variables are measured in the same unit) and that the partial derivatives, therefore, must satisfy the relations

$$\left.\begin{aligned} u &= \dot{V} \frac{\delta u}{\delta \dot{V}} + \dot{Q} \frac{\delta u}{\delta \dot{Q}} \\ \dot{V} \frac{\delta^2 u}{\delta \dot{V}^2} &+ \dot{Q} \frac{\delta^2 u}{\delta \dot{V} \delta \dot{Q}} = 0 \\ \dot{Q} \frac{\delta^2 u}{\delta \dot{Q}^2} &+ \dot{V} \frac{\delta^2 u}{\delta \dot{V} \delta \dot{Q}} = 0 \end{aligned}\right\} \qquad (16\ a)$$

Because of the last two relations, formula (15) can be written

$$u_T \approx u_0 - \frac{1}{2} \left[\frac{\dot{Q}_T}{\dot{V}_T} S_1^2 + \frac{\dot{V}_T}{\dot{Q}_T} S_2^2 - 2\, S_1 S_2 r \right] u_{12}$$

or considering (13)

$$u_T \approx u_0 - \frac{1}{2} \dot{V}_T \dot{Q}_T u_{12} s^2 = u_0 \left[1 - \frac{\dot{V}_T \dot{Q}_T u_{12}}{2\, u_0} s^2 \right] \qquad (17\ a)$$

th[illegible]

by [illegible] quantity u_0 on the other hand, is the maximum oxygen uptake which could be obt[illegible]

ventilation of $\dot{V}_T$ and a t[illegible]

variations in $\dot{V}/\dot{Q}$, and ot[illegible]

have been assumed throughout the analysis

An expression which is analogous to *(17a)* can be developed for the integral in *(7a)* because $F(\dot{V}\ \dot{Q})$ as defined by *(10a)* is also homogeneous in F, $\dot{V}$, $\dot{Q}$ and relations which are analogous to *(16a)* can therefore, be applied

$$\int F(\dot{V}\ \dot{Q})\,dL \approx F(\dot{V}_T\ \dot{Q}_T) - \frac{1}{2}\ \dot{V}_T\dot{Q}_T\ \frac{\delta^2 F(\dot{V}_T\ \dot{Q}_T)}{\delta\dot{V}\delta\dot{Q}}\,s^2 \qquad (18\ a)$$

From *(10a)* we find after some computation and taking the relations *(16a)* into consideration

$$\frac{\delta^2 F(\dot{V}_T\ \dot{Q}_T)}{\delta\dot{V}\delta\dot{Q}} - \dot{V}_T\dot{Q}_T\ \frac{(A-a)\dot{Q}_T - u_0]\,u_{12} + 2\,u_1u_2 - 2\,(A-a)\,u_1]}{[(A-a)\,\dot{Q}_T - u_0]^3}$$

This expression is inserted in *(18a)* expressions *(18a)* and *(17a)* are then used in *(7a)* and *(6a)* whereafter *(6a)* is subtracted from *(7a)* The result of these computations is

$$(x_A - x_a) \approx \frac{A\,(H-h)\,\dot{V}_T\dot{Q}_T u_1}{[(A-a)\dot{Q}_T - u_0]^2}\left[1 + \frac{\dot{V}_T u_1}{[(A-a)\,\dot{Q}_T - u_0]} - \right.$$

$$\left. - \frac{\dot{V}_T\dot{Q}^2{}_T u_1\ s^2}{4\,[(A-a)\,\dot{Q}_T - u_0 + \frac{1}{2}\ \dot{V}_T\dot{Q}_T u_{12}s^2]\ u_1}\right] s^2 \qquad (19\ a)$$

Actual calculations with ordinary values for the various variables and parameters show that the last term in the bracket of *(19a)* is numerically insignificant in relation to the two first terms (less than 0 05) For numerical computations we may therefore use the following simplified formula

$$(x_A - x_a) \approx \frac{A\,(H-h)\,\dot{V}_T\dot{Q}_T u_1}{[(A-a)\dot{Q}_T - u_0]^2}\left[1 + \frac{\dot{V}_T u_1}{[(A-a)\,\dot{Q}_T - u_0]}\right] s^2 \qquad (20\ a)$$

The variance s^2 of the variable t is the only quantity in this expression which depends on the variation of the ventilation blood flow ratio in the lungs The parameters A H and h are determined by the oxygen combining capacity of arterial blood the symbol a indicates oxygen concentration in mixed venous blood $\dot{V}_T$ and $\dot{Q}_T$ are total alveolar ventilation and total pulmonary blood flow u_0 is the maximum oxygen uptake which could be obtained with the same values of A H h a $\dot{V}_T$ $\dot{Q}_T$ and the same composition of inspired air but no variations of $\dot{V}/\dot{Q}$ and finally u_1 is

the partial derivative of $u(\dot{V}, \dot{Q})$ when $\dot{V} = \dot{V}_T$ and $\dot{Q} = \dot{Q}_T$. The quantities u_0 and u_1 are computed from (*8a*) with the values (*7a*) page 17 of the preceding paper for the constants c_1, c_2 and c_0.

It appears from (*20a*) and (*8a*) that $(x_A - x_v)$ depends on the ratio $\dot{V}_T/\dot{Q}_T$, but not on the absolute values of $\dot{V}_T$ and $\dot{Q}_T$, if the other variables and parameters in (*20a*) have specified values. The same is true for the expression within the bracket of (*17a*). Indirectly, the absolute values of $\dot{V}_T$ and $\dot{Q}_T$ may, however, be of importance, because low values of one or both of these variables may lead to a reduction in venous oxygen concentration, in order to maintain a given oxygen uptake. Venous oxygen concentration appears explicitly in (*20a*), and will also influence the values of c_1, c_2 and c_0 in (*8a*).

Numerical results from (*17a*) are given in the main section of the paper, for venous oxygen concentrations of 0.15 and 0.12, oxygen fractions of 0.21 and 1.00 in inspired air, and a barometric pressure of 760 mm Hg. The values (4a), based on 15 g hemoglobin per 100 ml blood, alveolar carbon dioxide tensions between 40 and 60 mm Hg and alveolar oxygen tensions above 60 mm Hg, will be used for the constants A, H and h. The following values were then computed for c_1, c_2 and c_0 according to (*7a*), page 17 of the preceding paper.

C_VO_2	F_IO_2	c_1	c_2	c_0
0.15	0.21	7.4	18.1	31.2
	1.00	1.0	14.6	1.0
0.12	0.21	6.8	11.7	13.1
	1.00	1.0	10.2	0.3

These values are inserted in equation (*8a*) from which

$$u_0 = u(\dot{V}_T, \dot{Q}_T) \qquad u_1 = \frac{\delta u(\dot{V}_T, \dot{Q}_T)}{\delta \dot{V}}, \qquad u_{12} = \frac{\delta^2 u(\dot{V}_T, \dot{Q}_T)}{\delta \dot{V} \delta \dot{Q}}$$

can be computed as functions of $\dot{V}_T$ and $\dot{Q}_T$. Three values, 0.5, 0.8 and 1.2 are finally chosen for the ratio $\dot{V}_T/\dot{Q}_T$.

Numerical results from (*20a*) are given for the same conditions, except for oxygen fractions of 1.00 in inspired air. The reason for this is that (*20a*) becomes inaccurate when $F_IO_2 = 1.00$, because numerator as well as denominator approach zero.

Formulas for carbon dioxide, corresponding to *(17a)* and *(20a)* for oxygen, can be obtained by similar calculations *Inspection of* equations (3a — b) and (5a — b), however, show that the formulas can be obtained directly from *(17a)* and *(20a)* by changing

$$H, h, u_0 \ u_1 \ u_{12} \text{ and } (x_A - x_a)$$

into

$$-K, -k \ -\dot{v}_0 \ -v_1 \ -\dot{v}_{12} \text{ and } (y_a - y_A)$$

This gives

$$v_T \approx v_0 - \frac{1}{2} \dot{V}_T \dot{Q}_T v_{12} s^2 = v_0 \left[1 - \frac{\dot{V}_T \dot{Q}_T v_{12}}{2 \ v_0} s^2\right] \qquad (17\text{ b})$$

$$(y_a - y_A) \approx \frac{B(k - K) \dot{V}_T \dot{Q}_T v_1}{[(B - b) \dot{Q}_T + \dot{v}_0]^2} \left[1 - \frac{\dot{V}_T v_1}{[(B - b) \dot{Q}_T + v_0]}\right] s^2 \qquad (20\text{ b})$$

Numerical results from *(17b)* and *(20b)* are given for venous carbon dioxide concentrations of 0 50 and 0 60 inspired air with a barometric pressure of 760 mm Hg and no carbon dioxide, the values *(4b)* for B, K and k, and the values 0 5, 0 8 and 1 2 for $\dot{V}_T/\dot{Q}_T$ The quantities v_0 v_1 and v_{12} are obtained from an equation which is analogous to *(8a)*, and given by *(6b)* page 21, with constants indicated on page 21 of the preceding paper

Summary

Approximate analytical relations between moderate variations in the ventilation blood flow ratio throughout the lungs mean alveolo capillary gas gradients and rates of gas exchange which can be obtained under specified conditions have been developed on a theoretical basis The most important parameters which influence the relations are the overall ventilation blood flow ratio venous gas *concentrations and composition of inspired air*

Numerical results obtained when atmospheric air is inspired and values within *normal ranges are inserted for the various para*meters, indicate that the oxygen gradients produced by a varying $\dot{V}_A/\dot{Q}_c$ are roughly 10 times greater than the carbon dioxide gradients The relation between the two gradients depends how ever, on the values of the parameters The carbon dioxide gradient will increase when there is a general *hyperventilation of the lungs*

The theoretical relations between arterio alveolo carbon dioxide gradients and differences between physiological and anatomical dead-space are discussed Numerical calculations indicate that these differences (measured in ml) will have approximately the same order of magnitude as the mean alveolo capillary oxygen gradient (measured in mm Hg) The size of physiological dead space, however, appears to increase in relation to the oxygen gradient with increasing predominance of hyperventilation in the lungs

Similar results were obtained for the effects of a varying $\dot{V}_A/\dot{Q}_c$ on the rates of gas exchange Generally, the oxygen uptake will be more impaired than the carbon dioxide output, but the detrimental effect on oxygen uptake will increase with increasing predominance of hypoventilation, and the detrimental effect on carbon dioxide output with increasing predominance of hyperventilation

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INTRODUCTION

A system for direct nervous control of the inner ear activity was discovered by RASMUSSEN (1946) Using the Marchi degeneration method he could trace a group of fibres from the superior olivary complex to the vestibulo-cochlear anastomosis in the contralateral inner ear In a later study RASMUSSEN (1953) could follow these crossed olivo-cochlear fibres to a region peripheral of the spiral ganglion of the cochlea

A multitude of recent experimental data suggest that the input from many afferent systems are controlled by centrifugal nervous pathways and that centrifugal control may be a general principle of action of the central nervous system For discussion and literature of such systems see GRANIT (1955) and HAGBARTH (1960) The most penetrating experimental studies of a centrifugal control system are those of GRANIT and his co-workers over the efferent gamma control of the muscle spindles (see GRANIT 1955)

Available data on the different systems controlling sensory input indicate that they may fulfill at least two different functions Studies by HERNÁNDEZ PEON and co-workers (for recent findings and reference to earlier literature see BACH y RITA, BRUST CARMONA, PENALOZA ROJAS and HERNÁNDEZ PEON 1961) suggest that centrifugal control may act as a gating mechanism, letting through the sensory input of highest significance in any actual situation and suppressing sensory input of secondary importance Another centrifugal control function is found in self regulating systems where the sensory input is controlled through a feedback loop activated by the sense organ itself A simple example of such a regulatory device is the pupil reflex to light which recently has been analyzed in terms of modern servotechnique (STARK 1959) The middle ear muscles seem to have a similar function of controlling the amount of stimulating energy to which the receptor is subjected

The function and the significance of the efferent system of the crossed olivo-cochlear fibres of RASMUSSEN remain largely obscure Some effects of direct electrical stimulation of these fibres have been studied, but nothing is known about how and from where they are activated GALAMBOS (1946) showed that electrical stimulation of the crossed olivo-cochlear fibres in the floor of the fourth ventricle depressed or abolished the action potential component of the round window response to a click GALAMBOS' findings were confirmed by FEX (1959) who showed that such electrical stim[illegible]

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RUE[illegible]

and[illegible]

when relatively weak electrical stimuli were applied without changing the action potential at the round window At higher stimulus strength the round window action potential was also abolished DESMEDT and MONACO (1960) showed that the effect on the action potential at the round window from electrical stimulation of the crossed olivo-cochlear fibres was blocked by strychnine and (DESMEDT and MONACO 1961) that the cochlear microphonic increase in response to such stimulation was also blocked by strychnine

The studies referred to seem to indicate that the crossed olivo cochlear fibres have an inhibitory effect upon auditory input at the inner ear level perhaps on certain key fibers in the eighth nerve essential for a cortical evoked potential (RUBEN and SEKULA 1960) The suppression of the synchronous action potential component of the round window response to sound cannot, however, be regarded as evidence that only inhibitory processes are at play It may well represent a summative effect of both inhibitory and facilitatory processes This question had to be approached by microelectrode analysis at the level of the primary auditory neurones (cf HAGBARTH and FEX 1959 for a discussion of the necessity of supplementing macroelectrode studies by microelectrode investigations)

Since according to current theories (cf DAVIS 1961) the relation between cochlear microphonics and action potential at the round window is that of receptor potential to generated neuronal activity the above mentioned findings of GALAMBOS (1956) and of FEX (1959) seemed to merit further analysis especially with regard to the site of action of the efferent system

In the present work I have approached the following problems concerning the function of the crossed olivo cochlear fibres 1) are these fibres acoustically activated and could they be found to participate in a feedback control 2) how is activity of the primary auditory neurons influenced by electrical stimulation of the crossed olivo cochlear fibres and 3) where is the site of action of these fibres Each of the three main problems will below be treated in a separate Chapter Thus Chapter I concerns a single fibre analysis of the efferent fibres It will be shown that such fibres are activated by sound In the next two Chapters the effects of these efferents are further studied In Chapter II this was done by recording from single primary auditory fibres These were identified according to a histological criterion Only inhibitory effects were found In Chapter III the action of crossed olivo cochlear fibres on some of the electrical events of the inner ear were studied in an attempt to gain some insight into the site of action of these fibres Earlier observations on the action potential were confirmed and the observations on the cochlear microphonic were extended It will also be shown that the resting potential at the round window was increased by stimulation of the olivo-cochlear bundle

CHAPTER I

SINGLE FIBRE ANALYSIS OF CROSSED EFFERENT FIBRES

In this series of experiments the response of the crossed olivo-cochlear fibres to sound has been analyzed, as part of the study of the function of these fibres

To enable better understanding of the procedures to be described it seems pertinent here to give a brief summary of RASMUSSEN's (1946, 1953, 1960) histological results obtained from cats, rats and opossums (1946) and from cats (1953, 1960)

The origin of the crossed olivo-cochlear fibres is confined chiefly to an area situated medial to the accessory olive and dorsal to the nucleus of the trapezoid body In this region small multipolar cells known as the retro-olivary group are found These cells are morphologically of the visceral efferent type, their dendrites intermingle with the fibrous plexus of the superior olivary complex and their axons are directed dorsally The crossed olivo-cochlear fibres rise through the brainstem to the floor of the fourth ventricle Before crossing the raphe they collect into a compact bundle beneath the facial genu The level of decussation is localized to the rostral border of the facial genu After crossing the fibres fan out but reconverge near the lateral side of the facial root At that point a few fibres enter in the medial angle of the medial vestibular nucleus and terminate about small cells in this nucleus The remaining fibres course laterally as a compact bundle to the dorsal border of the descending root of the fifth nerve where the crossed bundle joins the incoming vestibular root When leaving the ventrolateral margin of the fifth root the crossed olivo-cochlear bundle is broken up into three or more fascicles by fibres of the lateral trapezoid body and its latero-caudad course is changed abruptly At the point of emergence from the medulla the crossed olivo-cochlear fibres have a course parallel with that of the vestibular and facial roots At the level of the pia ring the crossed olivo-cochlear fibres are found to be located near the ventral surface of the vestibular root and can there be followed between the two divisions of the vestibular nerve More distally they are covered ventrally by the inferior vestibular division becoming more deeply situated in the vestibular nerve upon coursing peripherally When passing through the ganglion associated with the saccular nerve the scattered fibres tend to converge to the ventral extremity of the ganglion At that point they leave the

vestibular nerve to accompany the cochlear nerve as the vestibulo cochlear anastomosis of OORT (1918) The crossed olivo cochlear fibres join the cochlear nerve between the basal and second turn entering into a spiral formation at the external margin of the spiral ganglion and from there fibres are distributed peripherally throughout all turns towards the organ of Corti The fibres were found to extend beyond the spiral ganglion but could not be followed beyond a delicate spiral plexus situated near the margin of the osseous spiral lamina This was considered by RASMUSSEN as perhaps due to technical difficulties

RASMUSSEN (1960) added information about a connection of the crossed olivo-cochlear bundle with the ventral cochlear nucleus Fine fibres leave the main crossed olivo cochlear bundle to traverse the glial portion of the vestibular root in a dorsolateral direction Most of the fibres enter and terminate within the superficial cell layer of the ventral cochlear nucleus and the remainder enters the nucleus proper in a diffuse fashion The precise terminations of these fibres were not determined

The presence of an uncrossed olivo cochlear tract (RASMUSSEN 1960) has also been demonstrated Its fibres emanate from the S shaped olivary segment to pass dorsal and lateral to the ascending limb of the crossed component The uncrossed olivo cochlear fibres join the crossed olivo cochlear fibres from the opposite side lateral to the facial root In the same study he concluded that the total number of crossed olivo cochlear fibres in the cat is around 500 and that the uncrossed olivo cochlear tract has a fibre population about a fourth of this number A short description of the synaptic connections of the two fibre systems was presented The nucleus of origin of the homolateral efferents receives exclusively uncrossed connections from the cochlear nucleus while the cell groups associated with the origin of the crossed olivo cochlear fibres receive afferent connections predominantly if not entirely from the cochlear nucleus of the opposite side In other words the composite bundle of one side arises from nuclei that are connected with cochlear nucleus on the same side as that from which the bundle leaves the brain

Technique and Procedure

Selection of experimental animals

The experiments in this series were carried out on 47 cats weighing between 1 5 and 2 5 kg The animals were carefully inspected and chosen only if a) their behaviour indicated normal hearing b) they were free from signs of respiratory infections, c) they under otoscopic inspection showed a normal light reflex from the tympanic membrane d) their outer ears were free from

mite (*Otodectes cynotis*) Mites may cause pathological changes of the tympanic membrane and thus impairement of the cat's hearing Only one cat, 28 09 61, infected with this parasite has been used During the preparation of the animal, any pathological changes found in the middle ear or the inner ear under the dissecting microscope were used as a basis for elimination

The preparation of the animal for the experiment

Under ether anaesthesia the carotid arteries were ligated and the trachea and one of the femoral veins were cannulated When a femoral vein cannula had been inserted the administration of the ether was stopped, as a rule not more than 15 min after its initiation, and a short lasting barbiturate, Thiogenal (E Merck), was given in a dose of 10—20 mg/kg body weight A long incision was made in the midline over the skull, the skin and the muscles were reflected so that the external acoustic meatus could easily be exposed on both sides Tha cat s head was then firmly attached to the head holder, a modified Horsley Clarke instrument of the Labtronic's model Holes were drilled in the most lateral part of each temporal bone and in these holes pointed metal pins were inserted which served the same function as conventional ear pins. The eye holder was adjusted to match the height of the pins in the parietal bones A large opening was made in the parietal bone to the left of the midline and the cat was decerebrated precollicularly by suction

Using a temporal approach the left tympanic bulla was exposed and opened, the bony ring enclosing the tympanic membrane was removed and the left outer ear reflected Subsequent stages in the dissections were carried out with the aid of a binocular operating microscope (Zeiss Epiteknoskop), which during the latter part of the study had the Zeiss motordriven control for focusing operated by a foot switch The ossicles were removed but the tympanic tensor muscle was left The reflex contraction of this muscle to sound was used as a gross test of the function of the contralateral (right) ear during the experiment A small hook was inserted in the oval window towards the round window and the bony part between these openings removed by pulling the hook This opened the vestibule and the opening was widely enlarged upwards with a dental drill The floor and the medial wall of the vestibule were thus exposed and parts of these structures were removed with a fine drill The inferior part of the vestibular nerve was now accessible and was divided with finely pointed watchmaker's forceps To cite RASMUSSEN (1946, p 170 Under a dissection microscope Oort s anastomosis comes to view upon elevating the inferior division of the vestibular nerve from the groove formed by the basal and middle turns of the cochlear nerve It is also observed that the main bundle courses in a bed of connective tissue and

accompanies blood vessels near the bottom of the above mentioned groove and passes at right angles to the fibres of the basal turn (Fig 5)' The bed of *connective tissue and the blood vessels* mentioned by RASMUSSEN had to be reflected since they completely obscure the vestibulo-cochlear anastomosis in the living animal This was by far the most delicate part of the preparation The connective tissue was visualized under a magnification of 60 times and torn by the pair of finely pointed forceps Suction was applied with glass tubes drawn to tip diameters of 0 2—0 5 mm and flamed In spite of every precaution blood vessels were often torn with the connective tissue At this stage of the preparation even the smallest blood clot formed in the operative field could ruin the preparation In order to prevent formation of blood clots outside the vessels the preparation was repeatedly flushed with Ringer solution to which Heparin ('Vitrum) 3 I U /ml had been added This did not prevent closure of torn blood vessels although bleeding took longer time to stop than it would have done if external blood clot formation had been allowed This Heparin flusing technique proved to be of great importance for a successful preparation

The cerebellum was exposed and part of it removed over the fourth ventricle Under visual control stimulation electrodes were placed between the facial genua in the floor of the fourth ventricle The craniotomies and the drillholes were closed with dental cement to minimize pulsations of the brain The right pinna was amputated close to the bone ring enclosing the tympanic membrane, so that the reflected ear would not attenuate the sound stimuli to be presented

In a series of 20 animals stimulating electrodes were placed in the cochlear nerve peripheral to its basal turn Any bleeding that occured during the enlargement of the opening of the vestibular floor was controlled by gel foam (Spongostan, Ferrosan) Special care was taken not to contaminate the region of the vestibulo cochlear anastomosis with Spongostan

To prevent elevation of blood pressure, Thiogenal in doses of 10—20 mg/kg body weight was given i v just before the preparation of the left inner ear and before the neck muscles were detached Thiogenal was sometimes used at the end of the experiment to decrease movements in lively animals Flaxedil (May & Baker) in doses of 8—10 mg/kg body weight was occasionally used for the same purpose and the cat was then *artificially respirated*

Electrical stimulation

The stimulator used had an output resistance of 1 000 ohms and delivered square wave pulses but the pulse form was distorted by the insulation trans former Pulses of 0 6 msec were used either as single shocks or in trains

of tetanic bursts The burst duration, the pulse frequency and the pulse amplitude could be controlled independently of each other Stimulating current was not measured

For stimulation of the left cochlear nerve peripheral to its basal turn, two insulated copper wires with a diameter of 40 μ were used, the bared tips being 0 5 mm long and 0 5 mm apart from each other and from the recording site in the vestibulo cochlear anastomosis

For stimulation of the region where the olivo-cochlear fibres cross in the floor of the fourth ventricle electrodes were used which consisted of two enamelled platinum iridium wires The diameter of each wire was 0 2 mm, the interelectrode distance was 0 7—1 0 mm and the uninsulated 1 mm long pointed tips protruded from cuffs of polyethylene tubing that served as stoppers at the floor surface

In 5 animals histologic sections were studied of the stimulation site between the facial genua in the floor of the fourth ventricle Electrode tracts were found in or close to crossing bundles that seemed to fit the description given by RASMUSSEN (1946) for the crossed olivo-cochlear bundles

Acoustic stimulation

Acoustic stimulation was carried out with a sine wave generator (Philips' Model GM 2305 B) Its attenuator was used in either of two positions, separated by a step of the order of 40 db Finer gradations of the applied sound pressure at this part of the acoustic system were achieved with the aid of a separate attenuator provided with two series coupled divisions, one with 10 steps of 6 db each, the other with 10 3 db steps The connection between the tone generator and the attenuator was plugged and unplugged by hand when longer pips independent of the sweep circuit were used This technique was used during a great part of many of the experiments, since it was discovered that the transients presented no serious problem The output of the tone generator was fed into an electronic gate (Bruel & Kjaer type F1) when short pips were wanted The gating signal came from the stimulator and was synchronous with the signal that triggered the sweep to the oscilloscope Time of rise and decay as well as duration and amplitude of the pips could be varied independently of each other Pips from this gate were used for latency determinations and were presented to the cat through a hearing aid receiver that was mounted in one end of a 9 mm long tube, the other end faced the tympanic membrane at a distance of 2—3 mm. The electronic gate could be used only within a comparatively narrow sound pressure range and for many measurements another system was employed Occasionally acoustic "clicks" were produced by passing the short impulse, used for starting the stimulator,

through a power amplifier connected to the attenuator The characteristics of the "click" were not determined

The output from the attenuator was presented in an open field through a dynamic loudspeaker ("Philips" 7910) mounted in a 10 l closed back cabinet The loudspeaker was placed 30 cm from the exposed right tympanic membrane with its axis directed towards the membrane

The frequency response for the loudspeaker system was flat within ± 6 db between 200—5 000 c/s and showed a few dips of the order of 15 db between 5 000 and 7 000 c/s The magnitude and location of these dips differed with different positions of the loudspeaker within experimental conditions Above 7 000 c/s there was a downward slope of the order of 30 db/octave The loudspeaker's maximal output was 115 db *SPL* (*SPL*=Sound Pressure Level relative 0 0002 dyn/cm²) These figures were obtained under experimental conditions with a Bruel condenser microphone (No 4133) mounted 5 mm in front of the upper rim of the tympanic membrane to give a "parallel sound incidence' A few measurments of absolute sound pressures of stimuli given under experiments were done with the same instrument

With a "Rudmose" sound Analyzer (model RA 100) octave band analyses were made over intervals of one minute under experimental conditions The following figures for noise levels in the room were obtained 47 db, 50 db 40 db, 35 db, 27 db and less than 22 db *SPL* in respective frequency bands 37 5—75 c/s, 75—150 c/s, 150—300 c/s, 300—600 c/s, 600—1 200 c/s, and the three bands 1 200—2 400 c/s, 2 400—4 800 c/s and 4 800—9 600 c/s The figures for the frequencies 600—9 000 c/s were not high, but the background noise may have influenced the results

Recording technique

Capillary microelectrodes filled with 4 M NaCl and having a resistance, as measured in the preparation or in Ringer solution, of 20—40 megohms were used for recording The electrode mounted on a *micromanipulator* fixed to the Horsley-Clarke instrument, was connected by a cathode follower to an AC coupled differential amplifier The input of the cathode follower was 2 5 pf and the capacity of the micropipette was of the order of 5 pf The reference electrode was an Ag-AgCl wire placed on the rim of the opened round window The grounding Ag AgCl wire was put on the left temporal muscle with a piece of cotton soaked in Ringer solution The differential amplifier fed a split beam cathode ray tube, a pair of headphones and one of the two channels of a tape recorder ("Revox-Stereo", Model D 36) The *neuronal activity from* the preparation was monitored acoustically with the headphones and visually with the cathode ray tube On a few occasions the

cathode ray beam was photographed but as a rule the experiment was recorded on the tape recorder The second channel of the tape recorder was used for recording protocols, triggering signals, and the electrical equivalent of the stimuli used After the experiment the tape was played back into the cathode ray oscilloscope using one beam for the recorded neurophysiological events and the other for the recorded stimuli or for time calibration Sweeps were thus displayed and photographed having the same synchrony of events as in the original experimental setting This technique was advantageous in many ways There was no time lost during the actual experiment in deciding exactly how to display the events on the cathode ray oscilloscope The decision was made during repeated playbacks after the experiment, when the tape recorder's loudspeaker provided acoustical monitoring of the recorded signals or gave the protocol of the experiment Occasionally, it was found very convenient during the experiment to play back the tape on a Tektronix Type 522 Oscilloscope to check some finding and this could thus be done faster than if conventional photographic procedures had been involved The tape recorder was started and stopped by a foot switch while the input to the second channel was controlled by a manual switch When sweeps were played back and triggered from the tape s synchronous signals the first 0 1 msec of the sweeps were lost The frequency response characteristics of the tape recorder were such that no conclusions could be drawn from the shape of the potentials recorded No attempts were made systematically to record absolute size, shape or polarity of spikes

Stability of the preparation

Recording from single neuronal elements requires very stable conditions In an attempt to minimize the pulsations of the brain, all openings made in the skull were closed with dental cement, excluding of course the one for the left vestibulo-cochlear anastomosis As a further attempt to immobilize the left eighth nerve the intracranial part of the nerve and the region of the cochlear nuclei were covered by a firm gel of agar agar in Ringer solution However, the stabilizing effect of this procedure was doubtful A somewhat more effective method was used in a large group of cats The part of the cerebellum overlying the intracranial part of the eighth nerve and the region of the cochlear nuclei was sucked away Three or four insect pins with a diameter of 0 15 mm were inserted under visual control through a hole drilled in the temporal bone and into the intracranial part of the eighth nerve and the region of the cochlear nuclei until their tips were firmly anchored in the underlying bone In 20 cats the left cochlear nerve was electrically stimulated and so the eighth nerve and the region of the cochlear nuclei had to be

kept intact It was assumed that the critical fact was that the skull was not totally closed and then the size of the hole for the vestibulo cochlear preparation in the vestibule did not seem to matter Another main factor that would cause instability was pulsation of blood vessels close to the vestibulo-cochlear anastomosis Often the most apical fascicle of the anastomosis was immediately overlying a pulsating blood vessel and these structures could not be deflected from each other

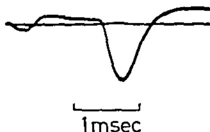
Even in the most stable preparations only few units could be recorded from for as long as 15 min Many units were kept for only 1—5 min and many for just a few seconds

Edema of the brain stem tended to herniate the intracranial part of the eighth nerve through the internal meatus with consequent interference with the blood supply of the preparation Edema was one of the big difficulties encountered with these preparations until urea administration became the routine A 30 % urea solution with 10 % inert sugar proved highly effective in preventing brain edema (see JAVID and SETTLAGE 1956, JAVID 1958) when given in three to four doses of 0 30—0 40 g/kg body weight at intervals of one to two hours

Control of the topography and histology of the fascicles of the vestibulo cochlear anastomosis

The vestibulo cochlear anastomosis was carefully dissected following each experiment The region was flushed with a 1 % solution of osmic acid which stained the nervous tissue black differentiating it from other tissues At the same time such procedures made the thin fascicles of the vestibulo cochlear anastomosis less liable to tear when manipulated It was found in some animals that the most apical fascicle had been hidden behind its neighbouring fascicle during the experiment The direction of the apical fascicle or fascicles was noted whenever it differed from what had been found to be the rule in preparations studied preceding this series of experiments In at least two of the cats a relatively large part of the apical fascicle took a radial course already 2 mm distal to the anastomosis which was exceptional Histological sections of the osmium stained vestibulo cochlear anastomosis were studied in 7 animals The 3—5 fascicles varied in width between 50 and 200 μ and were tightly packed with nerve fibres while no nerve cell bodies were identified The fibre diameters ranged from 1—5 μ with most of them being from 3—5 μ These figures were arrived at by measuring fibres using a measuring ocular The figures thus represent an approximation, but since they agreed with RASMUSSEN s findings (1946) that the main part of the vestibulo cochlear anastomosis consists of crossed olivo cochlear fibres with a diameter of 3 —5 μ there seemed no reason for having recourse to more elaborate procedures

Fig 1 Five superimposed action potentials recorded from a single crossed olivo-cochlear fibre in the vestibulo-cochlear anastomosis Single shocks were applied to the crossed olivo-cochlear bundle in the floor of the fourth ventricle about 15 mm from the recording site Negativity is upwards The initial, small positive deflection is the shock artifact The latency from the beginning of the chock to the inflection point of the initial positive phase of the action potential was 1 6 msec. Cat 20 06 61



Conduction of the experiment

If they could be separated at all from each other, the 3—5 separate fascicles of the vestibulo-cochlear anastomosis in the living animal were seen as 50—200 μ wide strands of nervous tissue, often poorly outlined against the background Through the approximate alignement of the shaft of the micropipette with the optical axis of the binocular operating microscope it sometimes was possible to control the direction of the microelectrode so that it could be placed in any division of the vestibulo-cochlear anastomosis the limiting factor being the poor visibility of the anatomical structures When the micropipette tip travelled the last few hundred microns towards the vestibulo-cochlear anastomosis the micromanipulator was controlled with the experimenters left hand his right hand kept the vestibulo-cochlear anastomosis free from fluid by micro suction while the microscope focusing was controlled with his left foot.

The physical contact between the microelectrode tip and the vestibulo cochlear anastomosis was signaled by a change in the noise from the monitoring headphones Immediately following contact a temporary 22 megohm

…ize the risk for blood clot formation (see p 10) Single shocks at the rate of 1—4 shocks/sec were applied to the site where the olivo-cochlear bundles cross in the floor of the fourth ventricle The shocks were synchronous with the signal triggering the sweep on the monitoring oscilloscope The tip of the microelectrode was slowly advanced As a rule a change of noise indicated that the tip was in contact with a nerve axon The micromanipulator was now handled with great care and as a rule an evoked potential soon appeared on the monotoring oscilloscope The tape recorder was immediately started and sometimes the potential was photographed from the monitoring oscilloscope (Fig 1) For several of the fibres,

recording conditions were such that nothing but these electrically evoked potentials could be taped perhaps with the addition of some activity aroused by the experimenter s voice a whistle or some other sound stimulus not *presented through the loudspeaker* Whenever possible, a unit *identified* by single shocks as a crossed olivo cochlear fibre was studied with acoustical stimuli from the loudspeaker and/or with electrical stimuli applied to the left cochlear nerve The fibre activity was monitored through both the head phones and the cathode ray oscilloscope and was recorded on tape Particulars for the different experimental situations will be given in the description of the results

The stimulus strength used as expressed in voltage output of the stimulator varied between 0 8 V and 8 V, 2 V being the strength generally used If single shocks of 4—8 V to the cochlear nerve did not activate a fibre this fibre was considered unexcitable by single shocks

There were no systematically recorded observations pertaining to the general condition of the animal In some experiments it was noticed that there was a prompt reflex contraction of the homolateral tympanic muscle to moderate sound presented to the contralateral ear even when very *strong* sounds were needed to evoke activity in the fibres studied This tympanic muscle reflex was taken to indicate that the right ear and the auditory pathways of the cat were still in good condition The activity of the right inner ear was not monitored in any direct way

Results

Criterion and introduction

Once it had been established that microelectrodes of comparatively high resistance *i e* with very fine tips had to be used single unit activity was recorded from every cat in which the vestibulo cochlear anastomosis was probed with a microelectrode Single unit activity in the anastomosis was driven by electrical stimulation of the floor of the fourth ventricle between the *facial genua The site where the crossed* olivo cochlear bundles intersect (RASMUSSEN 1946) in the midline between the rostral part of the facial colliculi was as a rule easily visualized so that the stimulating electrodes could be correctly placed For the identification of a fibre in the vestibulo cochlear anastomosis as a crossed olivo cochlear fibre it was considered a necessary and an adequate criterion that it could be electrically driven from the floor of the fourth ventricle (see Discussion p 52)

In the 47 cats of this experimental series 505 crossed olivo cochlear fibres were found Many of these were lost before they could be adequately tested

but 308 fibres were activated by acoustical stimulation of the contralateral ear or by electrical stimulation of the homolateral auditory nerve There were only 9 acoustically activated fibres in 7 cats that failed to meet the criterion for crossed olivo-cochlear fibres The high incidence of crossed olivo-cochlear fibres encountered 505 out of 514 seems surprising considering that the fibre population of the homolateral component in the vestibulo-cochlear anastomosis was judged by RASMUSSEN (1960) to be approximately one fourth that of the crossed component The explanation may lie in the fact that the crossed olivo-cochlear fibres were repetitively stimulated in the floor of the fourth ventricle which guided the probing with the microelectrode and biased the finding of crossed olivo-cochlear fibres in the vestibulo-cochlear anastomosis

When a fibre had been identified as belonging to the crossed olivo-cochlear bundle the presence or absence of resting activity was noted its response to acoustic stimuli was studied and the following questions were asked 1) Is there a graded response to sound stimuli 2) Can a threshold to sound stimuli at some particular frequency be well defined 3) Is the threshold very low as measured in db SPL 4) Is there a best frequency at which the threshold for stimulation of the fibre is lower than for any other frequency 5) Does the response to sound or to electrical stimulation of the cochlear nerve occur with a reproducible latency A positive answer to any one of these questions would indicate that auditory stimuli are adequate for activation of the crossed olivo cochlear fibres

Resting activity

Thirty five f bres out of 308 activated crossed olivo-cochlear fibres showed resting activity This activity which never occurred in bursts had a frequency of 1—10 imp/sec except in cat 28 07 61 in which 9 out of 13 crossed olivo-cochlear fibres had resting activity consisting of 10—20 imp/sec It may be significant that this cat was more lively than any other in the series The question as to a possible correlation between spontaneous activity and low threshold or high impulse frequency in the response to acoustical or electrical stimulation of the auditory nerve must be left open The scarcity of resting activity in a room with a noise level as given on p 12 was in itself of interest and also had the practical consequence that responses to sound as a rule could be studied without any obscuring background

Response to sound

A regular firing rate in response to sound stimulation has been a characteristic common to all crossed olivo cochlear fibres found n these experiments By

2

recording conditions were such that nothing but these electrically evoked potentials could be taped, perhaps with the addition of some activity aroused by the experimenter's voice, a whistle or some other sound stimulus not presented through the loudspeaker. Whenever possible, a unit identified by single shocks as a crossed olivo-cochlear fibre was studied with acoustical stimuli from the loudspeaker and/or with electrical stimuli applied to the left cochlear nerve. The fibre activity was monitored through both the head phones and the cathode ray oscilloscope and was recorded on tape. Particulars for the different experimental situations will be given in the description of the results.

The stimulus strength used, as expressed in voltage output of the stimulator, varied between 0.8 V and 8 V, 2 V being the strength generally used. If single shocks of 4—8 V to the cochlear nerve did not activate a fibre, this fibre was considered unexcitable by single shocks.

There were no systematically recorded observations pertaining to the general condition of the animal. In some experiments it was noticed that there was a prompt reflex contraction of the homolateral tympanic muscle to moderate sound presented to the contralateral ear, even when very strong sounds were needed to evoke activity in the fibres studied. This tympanic muscle reflex was taken to indicate that the right ear and the auditory pathways of the cat were still in good condition. The activity of the right inner ear was not monitored in any direct way.

Results

Criterion and introduction

Once it had been established that microelectrodes of comparatively high resistance, i.e. with very fine tips, had to be used, single unit activity was recorded from every cat in which the vestibulo-cochlear anastomosis was probed with a microelectrode. Single unit activity in the anastomosis was driven by electrical stimulation of the floor of the fourth ventricle between the facial genua. The site where the crossed olivo-cochlear bundles intersect (RASMUSSEN 1946) in the midline between the rostral part of the facial colliculi was as a rule easily visualized so that the stimulating electrodes could be correctly placed. For the identification of a fibre in the vestibulo-cochlear anastomosis as a crossed olivo-cochlear fibre it was considered a necessary and an adequate criterion that it could be electrically driven from the floor of the fourth ventricle (see Discussion, p. 52).

In the 47 cats of this experimental series 505 crossed olivo-cochlear fibres were found. Many of these were lost before they could be adequately tested



Fig 3 Response from a single efferent fibre to a continuous tone of 1st best frequency 2 900 c/s 30 db above threshold The tone outlasted the activity which stopped completely after 12 sec, second impulse in record D Between each of the records there are 2 6 sec intervals Cat 19 08 61

relative to the threshold The firing rate of the fibres of Fig 4 A had not reached a plateau at 60 db above threshold Most olivo-cochlear fibres tested in this respect gave graded responses to sound but several of them had a dynamic range of approximately 30 db or less It is not known whether 60 db is close to the maximum for the dynamic range of crossed olivo-cochlear fibres Only one fibre, Fig 4 B, was tested beyond this range and it showed a maximum firing rate at 66 db above threshold

The firing rates illustrated in Figs 2—4 are representative, but there were many fibres that could not be stimulated to discharge more than 10—20 imp/sec and a few that responded maximally to sound with only 2—5 imp/sec. Some fibres were stimulated with short, strong tone pips in an effort to make them fire pairs of impulses with short interspike intervals and in Fig 7 is shown how the spike interval decreases with increase of sound pressure The extremes found were spike intervals of 4—7 msec (unit 1 cat 24 05 61) and 7 msec (unit 3 cat 28 07 61) The highest firing rate at the beginning of a train of impulses was 9 impulses during 10 msec (unit 3 cat 04 08 61)

In response to loud continuous tones some fibres showed a pronounced decrease in firing rate with time, for the fibre in Fig 3 the activity stopped after stimulation for 12 sec. The resting activity that was present in some fibres was silenced for several seconds after loud tone pips of long duration In only one fibre (unit 7 cat 18 06 61) was there discharge of impulses after the end of the sound stimulus The after-discharge had a duration of more than 3 sec and was observed before and during the effect of Thiogenal

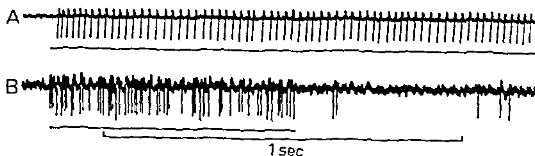


Fig 2 Responses to tone pips from a single efferent fibre (A) and from a primary auditory fibre (B) Note the regular firing pattern in A in contrast to the irregular pattern in B The records are from different experiments The durations of the sound stimuli are indicated by the horizontal bars under the records Cat 30 07 61 (A) Cat 08 12 61 (B)

contrast, primary as well as secondary auditory neurones often show an irregular pattern (GALAMBOS and DAVIS 1943, KATSUKI, SUMI UCHIYAMA and WATANABE 1958, ROSE, GALAMBOS and HUGHES 1959) Fig 2 illustrates this difference the response to sound is shown for a crossed olivo cochlear fibre in A and for a primary auditory fibre in B

Bursts, which are common to the pattern of primary and secondary auditory neurones were as a rule not seen in the crossed olivo cochlear fibres in response to sound stimuli Fig 3 represents the extreme in the way of a phasic component of the response Less exceptional at the other extreme is the finding shown in Fig 4 C1, unit 4 from cat 27 10 61, in which the first spike interval was longer than the next few intervals This kind of initial pattern was as common as the one shown in Fig 2 A, where the first spike interval was the shortest

The low firing rate also marked a difference between crossed olivo cochlear fibres on the one hand and primary and secondary auditory fibres on the other In these afferent fibres firing rates up to 1 000 imp/sec have been found (GALAMBOS and DAVIS 1943, TASAKI 1954) Fig 4 illustrating the relationship between stimulus and response, shows 4 fibres that reached firing rates of between 30—50 imp/sec at sound pressures of 60 db relative to their thresholds For plotting the curves in Fig 4 the thresholds were determined and the sound pressure was raised first in steps of 3 db and then in steps of 6 db Long tone pips of 1—2 sec duration or more were used with intervals somewhat shorter or of the same duration The number of impulses that occurred during the first second of the pips was determined on the filmed playback of the record and plotted against sound pressure expressed in db



Fig 5 Responses of a crossed fibre to loud tone pips of increasing duration. Note that the shortest tone pip in A did not evoke an impulse In B where tone pips are longer than in A a second impulse appears Tone frequency of tone pip 820 c/s Cat 26 07 61

Threshold determination

When a fibre had been identified as a crossed olivo-cochlear fibre the attenuator was set so as to give a moderately strong output of sound and the sine wave generator s frequency bands of 200—20 000 c/s were scanned If the fibre was activated by sound the frequency band giving the highest impulse frequency was scanned again this time at a much lower sound pressure level than the first time By stepwise production of sound pressure changes it was possible quickly to find the lowest sound pressure level within ± 3 db at which the fibre could be activated This lowest sound pressure level will be called the fibre s threshold and the sine frequency, at which the threshold was found, will be referred to as the fibre s best frequency The transients produced

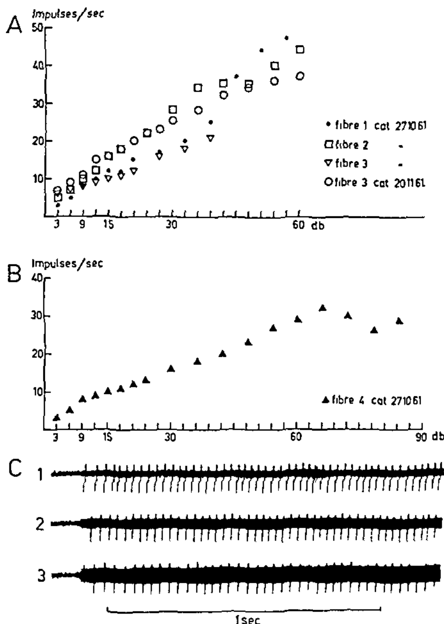


Fig 4 Relation between sound pressure and frequency In A and B the number of impulses fired during the first second of the presentation of a long sound pip has been plotted against sound pressure relative to the threshold of each of the fibres tested The four fibres represented in A never reached a plateau The fibre in B however, reached a maximal firing rate 66 db above its threshold The responses of this fibre to pips 66, 72 and 78 db above threshold are shown in C Note that the latency in the records of C increases with increasing sound pressure The beginning of the sound stimulation corresponds to a thickening of the base line Note also that the first inter-spike interval in C1 is longer than any one else in C1 Note also the regular firing rate

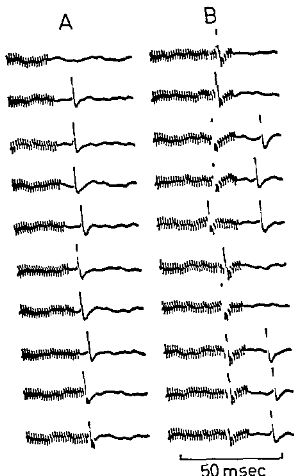


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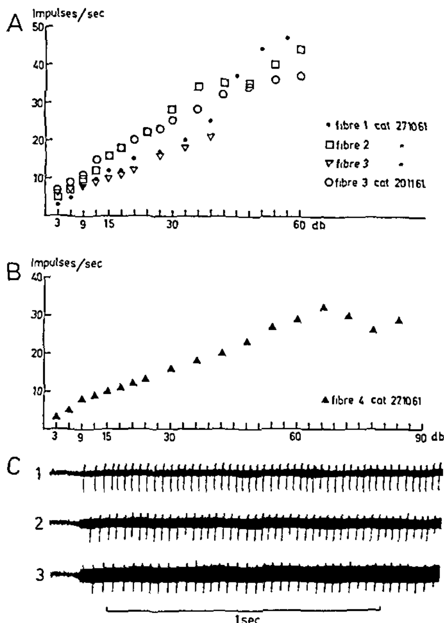


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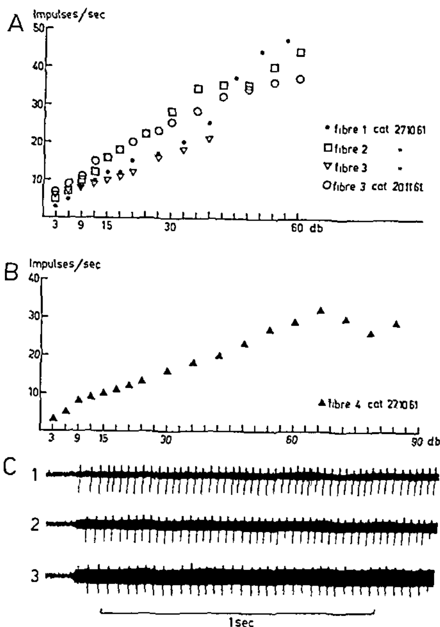


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Every unit tested in the way described had a well defined threshold within ± 3 db, provided that sound pressures markedly above threshold level had not been used for long durations Strong sounds often increased the threshold This may be exemplified by unit 2 in cat 27 10 61 The threshold was found at the attenuator setting − 57 db Sound pips were then presented with stepwise increase of sound pressure up to 0 db attenuation The pips were about 1½ sec long and the intervals between them were of the same order Some 20 sec after the last pip the threshold setting was − 48 db Pips up to maximal output were again given and 10—15 sec later the threshold setting was − 42 db The basis for the rise of threshold could be inherent in the crossed olivo cochlear nerve cell or it could be secondary to events in primary and secondary auditory neurones The following observation favours the latter alternative Rose et al (1959), working with single units of the cochlear nuclei found recovery times of minutes after moderate tones of short duration

Although absolute sound pressure levels were not always recorded it can safely be said that most of the units activated by sound did not respond below 40 db SPL and many of them not below 60—70 db SPL On the other hand a few units responded at very low sound pressure levels For two such units the sound pressures at the tympanic membrane were measured with a condenser microphone, for a third, the sound pressure was extrapolated from such a measurement made previously Thus unit 2, cat 10 11 61, was activated by a 4 000 c/s tone at 15 db ± 3 db SPL, unit 12, cat 14 11 61, by a 5 500 c/s tone at approximately 15 db SPL and unit 3 cat 20 11 61, by a 4 000 c/s tone at 25 db SPL

Best frequency and band width

Sometimes it could be decided in which fascicles of the vestibulo cochlear anastomosis the microelectrode tip had been placed (see above p 14 and p

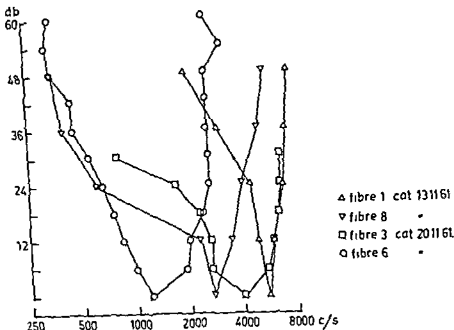


Fig 6 [illegible]
highest [illegible]
increasi[illegible] [illegible] thresholds of the neurones The absolute
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15) This made it possible to try to obtain information as to whether or not fibres running to the different parts of the cochlea responded preferentially to different frequencies

Most crossed olivo-cochlear fibres tested in the way described above had a best frequency (p 21) determinable within 10 % Table 1 offers data on all fibres which ran in the most basal or the most apical fascicle of the vestibulo cochlear anastomosis, as verified at section, and for which best frequencies had been determined There seemed to be a greater proportion of high best frequencies from the basal than from the apical fascicle The material, however, is too limited for this to be taken for more than an impression The highest and the lowest frequencies of sound stimuli activating each fibre was determined for different sound pressures The results in four fibres have been plotted as sound response curves in Fig 6 giving a graphical expression of the frequency range at different sound pressures The threshold at the best

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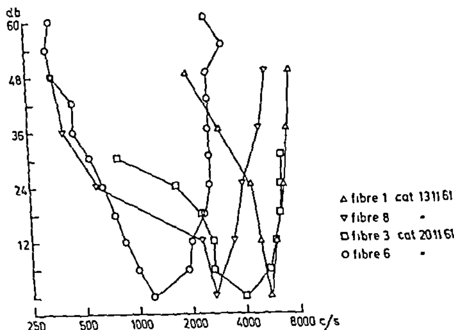


Fig 6 Diagrams have been plotted for four crossed efferents showing the lowest and highest tone frequencies at which these fibres responded when tested with stepwise increasing sound pressures, relative to the thresholds of the neurones The absolute values of the thresholds are not given. Note! the cut off at the high frequence side

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Table 1

cat	fibre	Fibres from the most apical fascicle			Fibres from the most basal fascicle		
		best frequency	db increase	lowest and highest frequency	best frequency	db increase	lowest and highest frequency
04 08 61	1	500					
	2	200					
	3				6 900		
19 08 61	1				2 800—3 000	30	800, 10 000
	2				2 700		
18 09 61	4	1 100					—, 10 000
20 09 61	1—3	2 500					
27 09 61	1	200					
12 10 61	4	2 500—3 000	60	500, 3 500			
25 10 61	1				1 200		—, 10 000
	2				1 100		—, 10 000
	3	1 100					
	4				3 000	30	700, 6 000
	5	900		200, 2 000			
	6	3 000		500, 3 300			
	7	1 300					
31 10 61	1	1 200	24	—, 2 000			
06 11 61	1	5—600					
	4	1 000					
	6	2 000					
10 11 61	2				4 000	18	2 000 4 600
	4	1 500					
	6	2 000					
13 11 61	1	5 500		—, 7 500			
14 11 61	1	3 500	12	350, 5 550			
	2				6 000		
	3				400		
	4	3 000					
	6	2 400					
	7	1 500					
16 11 61	2	3 000	18	700 4 000			
	7	3 500					
	10	900					
20 11 61	3	4 000	30	800, 6 500			
21 11 61	1	3 000					
23 11 61b	1	6 000					

frequency determined the coordinates for the minimum of a curve. Since inhibition at intensities high above thresholds has been reported to occur in neurones in the cochlear nuclei (ROSE et al 1959), the possibility should be mentioned that there may be several threshold frequencies for each frequency level The curves in Fig 6 represent at the chosen levels of sound pressure the lowest and highest values within which all possible frequency ranges must lie

Long tone pips were used as stimuli in these determinations No correction has been made for the loudspeaker system s deviation from an ideally flat response curve (see above, p 12)

The sound response curves of Fig 6 all show a cut off or a tendency to a cut off at the high frequency side and the fibres are similar in this respect to primary auditory neurones (TASAKI 1954, KATSUKI et al 1958) and to secondary auditory neurones (GALAMBOS and DAVIS 1943 TASAKI and DAVIS 1955 KATSUKI et al 1958, ROSE et al 1959) Often it was possible to determine the tone frequency range for a fibre at one sound pressure level only Data from such experiments are given in Table 1 The sound pressures applied corresponded as a rule to the maximal output of the loudspeaker system and could sometimes be expressed in db relative to the fibre thresholds Of the 9 identified olivo-cochlear fibres in basal fascicles, 6 were tested with high sound frequencies and 4 could be stimulated at 10 000 c/s while none out of 8 tested fibres in apical fascicles could be stimulated at such a high frequency Looking at the figures for cat 25 10 61 one finds that although there is overlapping of the best frequencies of the apical and basal fascicles the 3 fibres in the basal fascicle responded to much higher frequencies than did the two apical fibres

GALAMBOS SCHWARTZKOPFF and RUPERT (1959) claim that superior olive units respond to a limited region of the auditory spectrum and their response areas show with rare exceptions the triangular shape and steep drop-off at the high frequency border seen at the cochlear nucleus This generalization does not seem to hold for the single units of the present study Of the 6 units mentioned above from the most basal fascicle 2 responded to a sine wave frequency 3—4 times the best frequency and one to a sine wave frequency twice the best frequency Such wide band widths relative to the best frequency were also found in some fibres in the middle fascicles of the vestibulo-cochlear anastomosis while the fibres in the most apical fascicle showed more of a high frequency cut off

These findings indicate that crossed olivo cochlear fibres in the most basal fascicle in the vestibulo-cochlear anastomosis can respond to higher frequencies than can the fibres in the most apical fascicle They also suggest that fibres in the most basal fascicle can respond over a wider frequency range, expressed as multiples of the best frequency, than do fibres in the apical fascicle

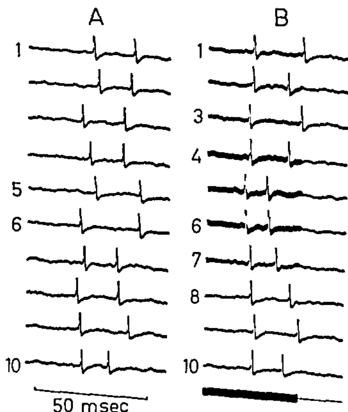


Fig 7 Responses of a crossed efferent to tone pips Tone pips were increased in strength between sweeps 5 and 6 in A, between sweeps 10 in A and 1 in B and between sweeps 3 and 4 in B Note that the latency is decreased with increase of sound pressure Note also decrease of interspike interval with increase in sound pressure Sound pressure was again lowered between sweeps 6 and 7 and between sweeps 7 and 8 in B This neurone was not activated by clicks Tone frequency of pips 2 250 c/s, pips were given at a rate of 4/sec The marking below B 10 indicates duration of pip Cat 19 07 61

Latency of response to sound

For the latency determinations, short tone pips of approximately best frequencies with rising times of 1—3 msec were presented The latency of evoked impulses to sound stimuli was defined as the time from the beginning of the sweep to the peak of the impulse

Latencies between 5 and 40 msec were found in 25 fibres stimulated with tone pips, only 5 of them had latencies below 10 msec Most units with longer latencies could not be stimulated with very short sound stimuli but behaved like the unit of Fig 5

Latency was found to decrease in several fibres with increase of sound pressure, as shown in Fig 7 Between the 5th and 6th sweep in Fig 7 A, the sound pressure of the pip was raised, the corresponding latencies decreased

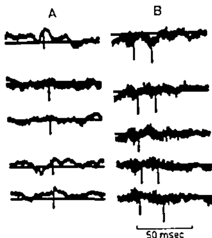


Fig 8 Responses of a crossed efferent fibre to tone pips Note that the latency decrased by a step when sound pressure was increased between A and B Pips were presented at the rate of one pip per second Cat 18 06 61

from 19—25 msec to 18—21 msec In Fig 7 B the sound pressure was increased twice Sweeps 1—3 and 4—6, with latencies of 15—16 and 13 5—15 0 msec respectively, show the effects of the two increases in sound pressure

In some fibres a stepwise change of latency was found, indicating that there are preferred intervals for firing an impulse In Fig 8 the intensity of the stimulating sound pip was greater in B than in A with a resulting latency shift of more than 15 msec It appeared as if this shift was due to the occurrence of a new spike coming up earlier, the position of original spike remaining unchanged

Contrary to the tendency illustrated in Figs 7 and 8 a prolonged latency was sometimes found when fibres were stimulated repeatedly at sound pressure levels at the upper end of their dynamic range The first spike interval then tended to be longer than the next few intervals These two points are illustrated in Fig 4 C 1—3

It may be significant that out of the 6 units of the exceptionally lively cat 28 09 61, 3 units had a latency less than 10 msec

Response to electrical shocks to the homolateral cochlear nerve

It has thus been shown above that crossed olivo-cochlear fibres can be activated by acoustic stimulation of the contralateral ear It would be pertinent for the question concerning an auditory activation of these fibres to investigate whether or not they could be activated also from the ipsilateral side Natural stimuli could not be used since the auditory function of the ipsilateral inner ear had been destroyed by the dissection The problem was therefore approached by electrical stimulation of the ipsilateral cochlear nerve

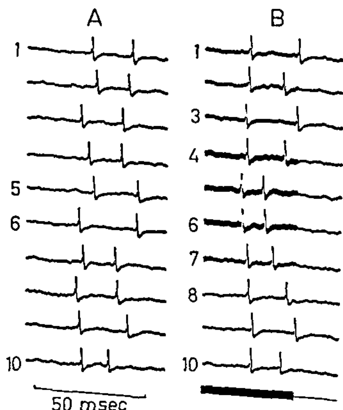


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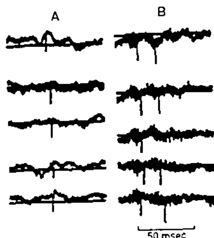


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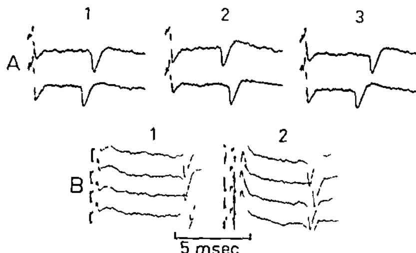


Fig 9 Responses of crossed efferent fibres in the vestibulo cochlear anastomosis to electrical stimulation of the ipsilateral cochlear nerve In A single shocks of two different strengths were given The size of the shock artifact seen to the left of the sweep *indicates the relative stimulus strength in each of the pairs of sweeps in A 1, A 2, and* A 3 The larger shock gives the shorter latency In B the effect of single (1) and paired (2) shocks are compared The latency is shorter in B 2 than in B 1 A and B are from the same *experiment* Cat 28 09 61

In 20 of the 47 animals, the cochlear nerve was stimulated electrically The procedures used were the same as described above (p 15) with the addition that stimulating electrodes were placed in the part of the cochlear nerve peripheral to the basal turn Single shocks and tetanic bursts at the rate of 1 500 shocks/sec were employed

The direct response to a single shock was never more than one impulse, and such an impulse had a latency in the range of 3 5—10 0 msec as measured from the beginning of the shock to the peak of the impulse The latency

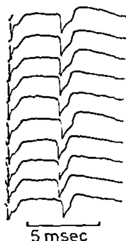


Fig 10 Responses of a crossed efferent fibre in the vestibulo-cochlear anastomosis to electrical stimulation of the ipsilateral cochlear nerve showing regular latencies Shock rate 4 shocks *per second* Cat 22 10 61

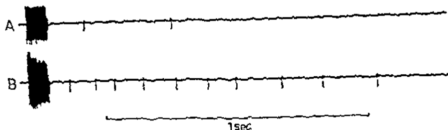


Fig 11 After discharges in a crossed efferent fibre in response to tetanic stimulation of the ipsilateral cochlear nerve with shock rate 250/sec. Between A and B the shock amplitude was increased from 2 V to 8 V Other stimulus parameters were unchanged Cat 16 11 61

depended somewhat on the stimulus strength used as seen in Fig 9 A where increased shock strength decreased latencies from 4 8—5 1 msec to 4 0—4 2 msec In Fig 9 B a change of stimulus from one schock to two shocks changed the latencies from 6 5—7 0 msec to 6 0—6 3 msec. Latencies to shocks of constant strength well above threshold were typically regular as shown in Fig 10 Sometimes an impulse appeared with a latency less than 1 5 msec that probably reflected current spread from the stimulating electrode to the olivo-cochlear fibre under study

In a few fibres repetitive stimulation to the cochlear nerve evoked not only direct responses but also after discharges which generally increased in rate and duration with increased stimulus strength and repetition rate The effects of increased stimulus strength on the after discharge are seen in Figs 11 A and B

In two fibres single shocks evoked a direct response only after the fibres had

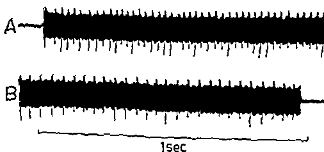


Fig 12 Beginning (A) and end (B) of a response of a crossed efferent in the vestibulo-cochlear anastomosis to tetanic stimulation during 10 sec of the ipsilateral cochlear nerve Record of 8 sec between A and B has been omitted The first interspike interval is longer than the few next intervals There is no initial burst. The firing rate is regular but has declined from A to B This neurone could not be activated by sound Cat 22 10 61

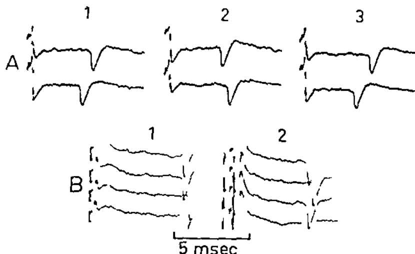


Fig 9 Responses of crossed efferent fibres in the vestibulo cochlear anastomosis to electrical stimulation of the ipsilateral cochlear nerve In A single shocks of two different strengths were given The size of the shock artifact seen to the left of the sweep indicates the relative stimulus strength in each of the pairs of sweeps in A 1 A 2 and A 3 The larger shock gives the shorter latency In B the effect of single (1) and paired (2) shocks are compared The latency is shorter in B 2 than in B 1 A and B are from the same experiment Cat 28 09 61

In 20 of the 47 animals the cochlear nerve was stimulated electrically The procedures used were the same as described above (p 15) with the addition that stimulating electrodes were placed in the part of the cochlear nerve peripheral to the basal turn Single shocks and tetanic bursts at the rate of 1 500 shocks/sec were employed

The direct response to a single shock was never more than one impulse and such an impulse had a latency in the range of 3 5—10 0 msec as measured from the beginning of the shock to the peak of the impulse The latency

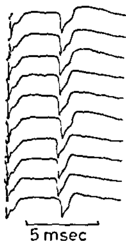


Fig 10 Responses of a crossed efferent fibre in the vestibulo cochlear anastomosis to electrical stimulation of the ipsilateral cochlear nerve showing regular latencies Shock rate 4 shocks per second Cat 22 10 61

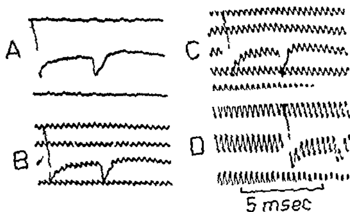


Fig 14 Sound conditioned responses of a crossed efferent in the vestibulo-cochlear anastomosis to single shocks applied to the ipsilateral cochlear nerve The conditioning sound was increased between each of the records A—D This neurone did not respond to uncond tioned shocks Note! the latency of the test responce decreased with increase in sound pressure Tone frequency 3 000 c/s Cat 21 11 61

Response to bilateral cochlear nerve stimulation

Out of 32 fibres activated by either mode of stimulation, 20 fibres were activated by both acoustic stimulation of the contralateral ear and electrical stimulation of the ipsilateral cochlear nerve A convergence of impulses from both cochlear nerves towards crossed olivo-cochlear neurones was thus demonstrated

To investigate whether signs of interaction between auditory inflow of the two sides could be found in crossed olivo-cochlear activity, the results of simultaneous stimulation of both cochlear nerves was studied in 4 cats (20 11 61, 21 11,61, 23 11 61a, and 23 11 61b)

It was found that acoustic stimulation increased the responsiveness to electrical stimulation in fibres that could not be activated by unconditioned single shocks Single shocks applied to the cochlear nerve were followed by single impulses in crossed olivo-cochlear fibres with latencies of 3 5—5 1 msec when the shocks were given during presentation of long tone pips In some fibres the acoustic stimulation was of subthreshold strength, one fibre could not be activated acoustically In one unit, which could be activated by either of the two modes of stimulation, subthreshold shocks given during sound stimulation were followed by impulses with a latency of 4 msec

In Fig. 14 is shown how an increase in sound shortens the impulse latency relative to single shocks This fibre could not be activated by unconditioned single

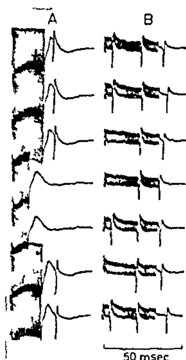


Fig 13 Responces of two crossed efferents in the vestibulo cochlear anastomosis to tetanic stimulation of the ipsilateral cochlear nerve The short bursts in A did not activate the neurone The longer bursts evoked an impulse In the record B from another experiment the impulses appeared with preferred intervals Note! the long latencies in both A and B Cat 28 09 61 (A) Cat 10 10 61 (B)

been conditioned with tetanic bursts an indication of post tetanic potentiation Fibres that showed post tetanic potentiation also showed prolonged after discharges with slow firing rates as in Fig 11 B

Repetitive antidromic stimulation with 1—3 shocks/sec, given in the floor of the fourth ventricle, increased the responsiveness of two fibres to single shock stimulation of the cochlear nerve, applied 0 3—1 0 sec after the series of conditioning shocks

The responses to tetanic stimulation of the ipsilateral cochlear nerve showed some of the characteristics of the effects of sound stimulation, described above, in that there was no initial burst and there was regular firing with low maximal rate as compared with primary and secondary auditory neurones Fig 12 illustrates these features as well as the fact that the first impulse interval was longer than the next few and that the firing rate was 41 impulses during the first second and 25 impulses during the last second of the 10 sec long tetanic burst

Sometimes with tetanic stimulation of the cochlear nerve long latencies were found that were of the same order as those described above for sound stimulation Fig 13 A shows how tetanic bursts of 20 msec duration evoked single spikes with latencies of 26—30 msec, while tetanic bursts of 11 msec were ineffective In B impulses appeared with preferred intervals and occasionally an impulse coming with a latency of 25 msec had no preceding impulse

CHAPTER II

EFFERENT INFLUENCE ON SINGLE PRIMARY AFFERENTS

In this section of the work effects of electrical stimulation of the crossed olivo-cochlear fibres on the activity of the primary auditory fibres have been studied. Since RASMUSSEN has described crossed olivo-cochlear connections with the cochlear nucleus as well as with the cochlea proper, it was considered necessary to have a valid criterion for differentiating primary from secondary auditory neurones.

The value of a criterion based on short latencies to sound seems questionable in view of the results of KATSUKI, SUMI, UCHIYAMA and WATANABE (1958) who found lantencies ranging from 1—5 msec in the dorsal cochlear nucleus of cats. TASAKI (1954) reported time intervals from 1.1—1.3 msec between the start of the microphonic response and the earliest single primary auditory fibre response to strong 8 000 c/s pips (in guinea pig). In the absence of a satisfactory electrophysiological basis for identifying primary auditory neurones a histological criterion was established: the following studies were pertinent in this regard.

ALEXANDER and OBERSTEINER (1908) described findings of glial islands in the cochlear branch of the eighth nerve in man peripheral to the glial septum. In longitudinal sections of the eighth nerve stained by the Nissl method a light central region can be differentiated from a dark peripheral region. The transition zone is concave centrally and represents the glia septum (ALEXANDER and OBERSTEINER 1908). Somata of secondary auditory neurones are found central to this septum: the small grain cells are located close to the septum while the large multipolar nerve cells are more centrally placed (see RAMON y CAJAL 1952).

TARLOV (1937) confirmed the findings of ALEXANDER and OBERSTEINER in man and stated that "confirmatory observations were made on nerves from monkeys, cats and rabbits — — —".

GALAMBOS and DAVIS (1948) described the presence of nerve cell bodies in histological sections of the intracranial part of the eighth nerve of cats.

CONTU (1958) referred to a work by CONTU, NEVES PINTO and SANTOS (1956) in which by using a silver method based on work by RAMON y CAJAL and DE CASTRO the authors found large multipolar nerve cells in the cochlear

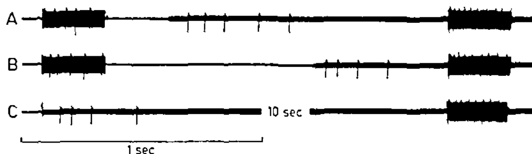


Fig 15 This figure shows the responce of one crossed efferent in the vestibulo-cochlear anastomosis to tetanic stimulation of the ipsilateral cochlear nerve To the left in A and B are shown the responses to unconditioned tetanic bursts, to the right in A, B and C are shown the responses to tetanic bursts conditioned by sound Note! the increased number of impulses when the tetanic bursts were sound conditioned No tetanic burst was given in C before the one illustrated Note that ony 4 to 5 impulses were evoked by the tone at its beginning The frequency of the conditioning sound was 2 500 c/s Cat 21 11 61

shocks The increased responsiveness of a fibre to bilateral cochlear nerve stimulation is illustrated in Fig 15 As shown in A and B of this figure 6 nerve impulses were evoked by a tetanic burst without a background of sound stimulation and 9—10 impulses were fired when the burst was given concomitant with sound Fig 15 C illustrates that the facilitating effect of sound remained after 10 sec of sound stimulation, the fibre fired a few impulses only at the beginning of the pip There was also a change of latency of the first impulse in the burst, from 5 1 msec without the tone to 4 6 msec when the tone was presented

Increase of responsiveness was demonstrated in 11 out of 12 fibres In the one exception the fibre could be activated by sound but not by electrical stimulation alone and single shocks or tetanic bursts of shocks applied to the cochlear nerve were not followed by impulses in any constant time relationship when sound was presented together with the shocks

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3

division of the eighth nerve in cat It was not stated how far peripherally the cells were found or of what size they were They were believed to be secondary auditory nerve cells

In the present work the cochlear division of the eighth nerve was investigated histologically The findings — to be described — led to the conclusion that *no secondary auditory neurones occur* peripheral of the main glial septum in the nerves studied Accordingly (see below) criteria were developed for locating the recording microelectrode to this site

Technique and Procedure

Selection of experimental animals

The experiments in this series were carried out on 12 cats weighing between 1 8 and 3 2 kg They were chosen by the criteria described above (see p 8) Animals in which blood collected in the cochlea during manipulations of the eighth nerve were discarded In order to determine the presence of blood in the cochlea it was found practical to have the bulla open during the experiment to permit inspection of the inner ear through the round window membrane

The preparation of the animal for the experiment

The animals were decerebrated stimulating electrodes were placed in the floor of the fourth ventricle and the bulla was exposed as described above (p 9) In cat 08 12 61 a deep incision was made to cut the olivo cochlear bundles 2 mm to the right of the stimulating electrodes in the floor of the fourth ventricle In 6 animals round window electrodes were used for monitoring inner ear activity and in 2 cats the middle ear muscles were divided

The region of the left cochlear nuclei was freed from the overlying part of cerebellum The final removal of cerebellar substance by suction at the internal auditory meatus was done under the microscope with glass tubes drawn to tip diameters of 0 5—1 0 mm and flamed The Heparin flushing technique (p 10) proved to be of importance here too for a successful preparation Of even greater importance was the use of a 30 % urea solution with 10 % inert sugar to prevent edema of the brainstem (see p 14) Such edema always changed the topographical relationships between the internal auditory meatus and intracranial part of the eighth nerve making the successful approach with a microelectrode to the eighth nerve difficult or impossible The third crucial technical device for this work was to anchor the intracranial part of the eighth nerve to the bone with insect needles (see above p 13) which gave relatively stable recording conditions Gel foam was used to stop bleedings in this part of the preparation and sometimes for closing an opened petrous sinus

Stimulating technique

The stimulator and stimulating electrodes were described above (p 10) as were also the acoustical equipment and the noise level of the room (p 12)

Recording technique

Capillary microelectrodes filled with 4 M NaCl or with 3 M KCl and having a resistance, as measured in the preparation, of 10—40 megohms were used for recording from single auditory neurones The activity was monitored and recorded as described above (p 12) Chlorided silver wires were used to record cochlear signals from the round window They were connected to an AC-coupled differential amplifier feeding the second beam of the split beam cathode ray tube

Control of the topograhy and histology of the cochlear division of the eighth nerve relative to the position of the micropipette tip

For recording the micropipette was inserted into the intracranial part of the eighth nerve close to the upper margin of the internal auditory meatus It was then advanced towards the apex of the modiolus at a direction almost tangential to the eighth nerve Recording was started when the electrode tip was considered to be peripheral to secondary auditory neurones Branchings of surface vessels were used as references for localizing the point where the electrode tip had entered the intracranial part of the eighth nerve

The length of tissue transversed by the electrode tip was approximated on the scale of the micromanipulator Such an approximation is defended by the following argument Had the microelectrode pushed with it the nerve the action potentials recorded would probably have changed as if the electrode tip had slowly been pushed further Such changes of the action potentials were not the rule

When the experiment was finished, the micropipette was withdrawn and its insertion point was marked with a finely ground tip of platinum wire dipped in Indian ink The holder and manipulator were left in position thus maintaining the direction of the micropipette The animal was then killed with Thiogenal The eighth nerve was dissected with care being taken not to change its topographical relationship to the remaining bone structures during removal of tissues over the lateral side of the nerve Following the dissection the tip of the micropipette was lowered again and the binocular operating microscope was placed so that a right angle was formed between the optical axis of the microscope and the long axis of the micropipette With the microscope in this position and with the aid of a measuring ocular distances along the eighth nerve were measured as if they had been projected on the pathway of the

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microelectrode tip The eighth nerve was marked with Indian ink at a given distance of 2 or 2 5 mm from the first ink spot A piece of cotton soaked in a 10 % formalin solution was then placed upon the eighth nerve and left there for about one hour Then one transection was made at right angles to the direction of the micropipette central to the Indian ink spot in the intracranial part of the eighth nerve and another transection was made peripheral to the second ink spot The cochlear division of the eighth nerve together with the cochlear nuclear region between the two transections was removed for fixation in 10 % formalin Histological sections, 12 μ thick, were cut parallel to the transection in the cochlear nuclear region, and stained for Nissl substance

The two ink spots were easily identified in the stained sections of the cochlear nerve Preliminary experiments demonstrated that shrinkage of the preparation was uniform The thickness of a histological section could thus be expressed in terms of distance travelled by the tip of the micropipette The distance was known from the settings of the micromanipulator and in this way the recording sites of the microelectrode could be determined approximately Edema was the main source of error since it not only made the choise of site for insertion of the pipette tip difficult but sometimes also made it impossible to identify this site at the end of the experiment Blood clots could have the same effect With the introduction of urea to prevent edema (see above) and Heparin locally to prevent blood clotting (see above) these difficulties were removed from most experiments Still it was considered safe to allow for a wide margin of error and many of the observations reported were done with the microelectrode tip advanced more than 1 5 times the distance that seemed sufficient to satisfy the criterion for recording from primary auditory neurones (see below, p 37)

It should be stressed that the procedures described above precluded recording from afferents from the most basal part of the cochlea The primary neurones from the basal part of the cochlea can be reached from the internal auditory meatus with a straight micropipette but the relatively short distance from the region of the secondary auditory neurones to the bony cochlea gives a narrow margin of safety A technique with steel microelectrodes permitting exact marking (GREEN 1958 modification suggested by GREEN in BROWN and TASAKI 1961) would be advisable in work with primary auditory neurones from the basal turn, if recording from these neurones is feasible with such electrodes

Conduction of the experiment

The tip of the micropipette was placed in the region of the cochlear nuclei under microscopic control Fine glass tubes with tip diameters of 0 2—0 5 mm were used for sucking away the cerebro spinal fluid from the dorsal surface

of the intracranial part of the eighth nerve at the internal auditory meatus The input cathode follower was temporarily connected to ground over a grid leak of 22 megohm The change of noise in the headphones marked the contact between the cochlear nuclei region and the tip of the electrode The setting of the micromanipulator was registered and the resistance of the pipette was measured The pipette was advanced about 2 5 mm in one minute and if the pipette resistance did not change the actual recording began From now on the animal was curarized with Flaxedil, 6—10 mg/kg body weight

Results

Histological features pertinent to recording from primary auditory neurones

With respect to the possibility of recording from primary auditory neurones as differentiated from secondary neurones (see above, p 33) by the technique described above (p 35), the histological findings were promising Multipolar nerve cells, of the variety found central to the main glial septum, were never seen peripheral of this septum in the 36 auditory nerves studied The transition from the central to the peripheral portion of the cochlear branch of the eighth nerve is sometimes irregular, presenting gaps which allow tongues of glial tissue to project peripherally, findings which confirm those of ALEXANDER and OBERSTEINER (1908) and of TARLOV (1937)

The distance from the insertion site of the microelectrode in the intracranial part of the eighth nerve to the most peripheral part of the main glial septum was found to vary between less than 1 mm to more than 3 5 mm The main part of this variation was in the length of the central part of the cochlear division of the eighth nerve, as measured from the somewhat poorly defined upper margin of the internal meatus Because of this variation each eighth nerve recorded from was studied histologically

Criterion

In this study nerve impulses activated by sound have been recognized as coming from a primary auditory neurone only if the recording site of the microelectrode was localized to the part of the cochlear division of the eighth nerve that was peripheral to the main glial septum By this criterion, however, the efferents were not excluded

Several groups of fibres were found which could not be activated by sound and which had a resting activity that was much more regular than that of auditory fibres This regular discharge could sometimes be accelerated by tetanic electrical stimulation of the floor of the fourth ventricle, but there was never a one to-one relationship between shocks and impulses, and no response

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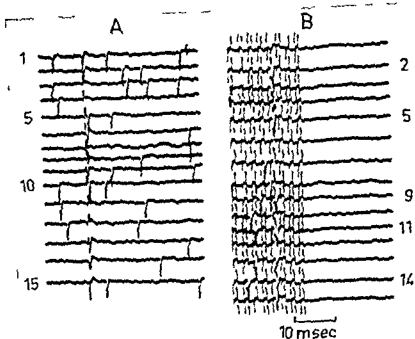


Fig 16 The effect of tetanic stimulation of crossed efferents on the *resting activity* in a primary auditory fibre Clicks presented with a delay of 10 5 msec relative to the beginning of the sweeps triggered one impulse in most of the sweeps in both A and B In addition tetanic bursts were triggered simultaneously with the sweeps in B The resting activity before the click is represented by 6 impulses in A and by 5 impulses in B The number of click evoked impulses in A and B is approximately the same Note that no impulses are seen in B *after the click* evoked ones Decerebrate cat curariezd with Flaxedil

Best frequency of primary auditory fibre 10 000 c/s Distance from electrode insertion point to most peripheral part of glial septum 1 6 mm recording from a depth of 2 3 mm. Cat 09 01 61

Fig 17 B the clicks were delayed 17 5 msec relative to the start of the burst and under these conditions three out of four tetanic bursts did inhibit the click response Tetani initiated less than 15 msec before the click failed to inhibit its response The minimal duration of stimulation required for inhibition in this and other fibres was of the same order when the same stimulus frequency was used (425 shocks/sec)

That considerable time is needed for building up full inhibition of the activity in a primary auditory neurone is illustrated in Fig 18 Trains of shocks at the frequency of 250 shocks/sec were triggered with sweeps The sweeps

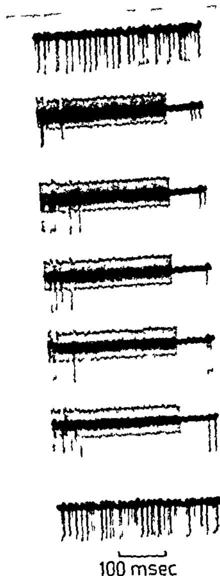
to single shocks as monitored by cathode ray tube and the headphones In spections after such experiments always revealed that the direction of the micropipette had been inadequate for recording of primary auditory fibres and that the tip of the microelectrode had been in the vestibular portion of the eighth nerve No systematic analysis of these findings was performed It was possible that efferent vestibular fibres (Rasmussen and Gacek 1958 Gacek 1960) were stimulated giving a change of activity in vestibular afferent fibres

Effect of electrical stimulation of crossed olivo cochlear fibres on activity in primary auditory neurones

In this material consisting of 56 primary auditory fibres there was not one instance of facilitation of resting or sound evoked activity by electrical stimu lation administered to the floor of the fourth ventricle In all but 4 of the 56 fibres inhibition was found when the floor of the fourth ventricle was electri cally stimulated These 4 fibres had no other recorded characteristics by which they could be differentiated from the other 52 fibres The last two cats in this study gave the most stable recording and stimulating conditions, 9 primary auditory fibres were studied in one and 11 in the other In each of these two experiments great differences in sensitivity to olivo cochlear stimulation were observed even between two consecutively studied fibres However the inter esting question if there are different classes of primary auditory fibres relative to sensitivity to crossed olivo cochlear inhibition has not been studied

Resting activity was found in many fibres In several instances it was not possible to decide whether such activity was spontaneous or in fact caused by the noise in the room (see p 12) However when it appeared in fibres with thresholds 20—40 db above the noise level of the room it was concluded that it was true spontaneous activity This activity could be inhibited by electrical stimulation of the crossed olivo cochlear fibres if sound evoked activity was inhibited by such stimulation Inhibition of resting activity is illustrated in Fig 16 One click a second against a background of resting activity was presented with a delay of about 10 5 msec relative to the signal triggering the sweep The click response consisted of one impulse In B a brief tetanus of shocks to the floor of the fourth ventricle was initiated at the beginning of each sweep The resting activity following the click responses was inhibited but click evoked impulses and impulses preceding the clicks were still found

Fig 17 shows inhibition of click evoked impulses The click was just strong enough to give at least one impulse In Fig 17 A the clicks were presented with a delay of 15 5 msec relative to the signal triggering the sweep and the tetanic burst The first tetanic burst gave an inhibition the others did not In



F g. 18 Effect of tetan c electrical s imulat on of crossed efferents on tone evoked [illegible]

after cessat on of stimulat on

Same neurone as n f g 16 same st mulat ng parameters expect for duration of tetan c burst

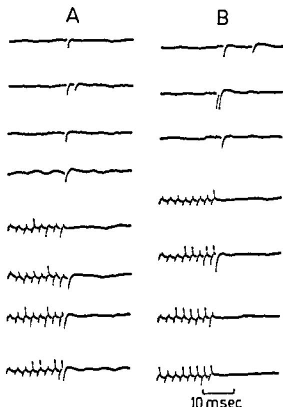


Fig 17 Effect of tetanic stimulation of crossed efferents on click evoked activity in a primary auditory fibre The click was chosen just strong enough to give at least one impulse The tetanic burst was triggered simultaneously with the sweep, the click was delayed less in A than in B Note that the first tetanic burst in A inhibited the click response Note that in B the click responce is inhibited 3 times out of 4

Best frequency of the primary afferent was 10 000 c/s Distance from electrode insertion point to most peripheral part of glial septum 1 6 mm, recording from a depth of 2 3 mm Cat 09 01 61

were consecutive and occurred at 2 sec intervals The auditory fibre was stimulated with a tone 18 db above the fibre threshold It is seen that the inhibitory effect of tetanic stimulation needed up to 75 msec to be fully developed and inhibition lasted for 70 msec after cessation of the tetanic stimulation

Another characteristic of the inhibition induced by electrical stimulation of the olivo cochlear bundle was that it tended to dissipate during prolonged stimulation With adequate choice of parameters of stimulation, dissipation of inhibition could be found after some tenths of a second This is illustrated in Fig 19 The primary auditory fibre was stimulated with sound at more than 5 db but less than 10 db above threshold and then with 5 db increases in sound pressure between each tetanic burst applied at 2 5 to 3 0 sec intervals After the period of maximal inhibition (Fig 19 D) nerve impulses began to appear 230—270 msec following the beginning of the tetanic burst

The degree of inhibition varies with stimulus strength and increases with the shock frequency up to 250 or 425 shocks/sec Such frequencies were more effective than 150 or 800 shocks/sec Fig 18 demonstrates that activity

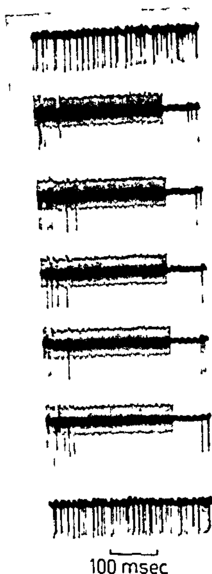


Fig 18 Effect of tetanic electrical stimulation of crossed efferents on tone evoked activity *in a primary aud tory neurone The primary afferent is stimulated with a tone* of its best frequency 10 000 c/s at 18 db relative to its threshold. Tetanic bursts were triggered simultaneously with the sweeps, Note that stimulation of 75 msec was needed to yield full inhibitory effect and that there is a post stimulatory inhibition of 70 msec after cessation of stimulation

Same neurone as in fig 16 same stimulating parameters expect for duration of tetanic burst

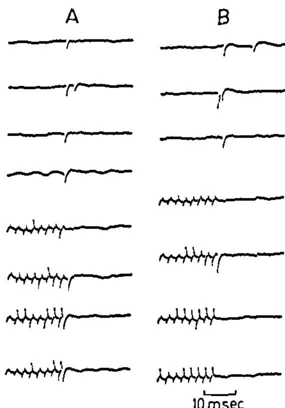


Fig 17 Effect of tetanic stimulation of crossed efferents on click evoked activity in a primary auditory fibre The click was chosen just strong enough to give at least one impulse The tetanic burst was triggered simultaneously with the sweep, the click was delayed less in A than in B Note that the first tetanic burst in A inhibited the click response Note that in B the click responce is inhibited *3* times out of *4*

Best frequency of the primary afferent was 10 000 c/s Distance *from electrode insertion point to* most peripheral part of glial septum 1 6 mm, recording from a depth of 2 3 mm Cat 09 01 61

were consecutive and occurred at 2 sec intervals The auditory fibre was stimulated with a tone 18 db above the fibre threshold It is seen that the inhibitory effect of tetanic stimulation needed up to 75 msec to be fully developed and inhibition lasted for 70 msec after cessation of the tetanic stimulation

Another characteristic of the inhibition induced by electrical stimulation of the olivo cochlear bundle was that it tended to dissipate during prolonged stimulation With adequate choice of parameters of stimulation dissipation of inhibition could be found after some tenths of a second This is illustrated in Fig 19 The primary auditory fibre was stimulated with sound at more than 5 db but less than 10 db above threshold and then with 5 db increases in sound pressure between each tetanic burst applied at 2 5 to 3 0 sec intervals After the period of maximal inhibition (Fig 19 D) nerve impulses began to appear 230—270 msec following the beginning of the tetanic burst

The degree of inhibition varies with stimulus strength and increases with the shock frequency up to 250 or 425 shocks/sec Such frequencies were more effective than 150 or 800 shocks/sec Fig 18 demonstrates that activity

evoked by a continuous tone more than 20 db but less than 25 db above the fibre threshold was totally inhibited during 240 msec by electrical stimulation of the crossed olivo-cochlear fibres In another fibre the activity evoked by a continuous tone 24 db above threshold was abolished for some tenths of a second in response to such stimulation

Rebound after inhibition was never observed, suggesting that the inhibitory effect is pure

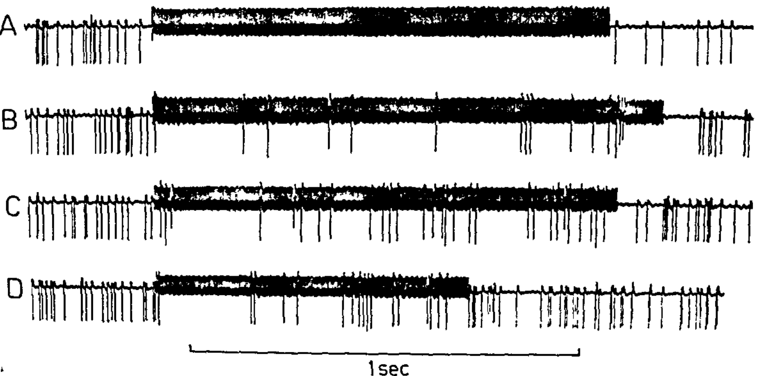


Fig 19 Effect of tetanic electrical stimulation of crossed efferents on tone evoked activity in a primary auditory neurone The primary afferent is stimulated with a tone of its best frequency 950 c/s In A the sound pressure was kept at a constant level between 5 and 10 db relative to the threshold of the fibre Between each record the sound pressure was increased with 5 db Tetanic bursts were applied to the efferents Note the total inhibition in A and some post stimulatory inhibition after cessation of stimulation Note that in B, C and D there was a dissipation of inhibition from efferent stimulation after about a quarter of a second

Middle ear muscles cut Distance from electrode insertion point to most peripheral part of glial septum 1 7 mm, recording from a depth of 2 4 mm Cat 06 12 61

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CHAPTER III

COCHLEAR POTENTIALS CHANGED BY EFFERENT STIMULATION

The results of GALAMBOS (1956) suggest that the crossed olivo cochlear fibres act somewhere in the region where acoustic energy is transformed into nerve impulses The result of the many studies concerned with this transmission mechanism (see DAVIS 1961) are used in the present work for a further analysis of the site of action of the system under study The action of strychnine as reported by DESMEDT and MONACO (1959, 1960) as well as the results to be described here add further information as to how efferent endings may be functionally localized

Technique and Procedure

Selection of experimental animals

The experiments in this series were carried out on 80 cats weighing between 1 5 and 3 5 kg They were selected by the criteria described above (p 8)

The preparation of the animal for the experiment

Most of the animals were decerebrated but some were kept under narcosis with Thiogenal Stimulating electrodes were placed in the floor of the fourth ventricle and the bulla was exposed as described above In several animals the middle ear muscles were divided

Stimulating technique

The technique and the equipment used for electrical as well as for acoustical stimulation have been described above (pp 10 and 11)

Recording technique

Chlorided silver wires with a diameter of 0 1—0 2 mm were used as electrodes one electrode was placed on or near the round window and another was located a short distance away as a reference electrode The wires were enclosed in thin polyethylene tubing and, for stabilization, the tubes and wires were knotted and embedded in dental cement on the rim of the bulla The bulla was closed with dental cement in most of these animals

In 3 cats pipettes filled with a saturated KCl solution and linked through a KCl agar bridge to calomel electrodes were used for recording The tip diameters of the pipettes were of the order of 0 1—0 2 mm One pipette was placed on the rim of the round window, touching but not stretching the round window membrane The reference electrode was placed on the medial wall of the bulla. The calomel half cells (Beckman fibre junction reference electrode No 39270) were connected to the input cathode followers of a DC-coupled differential amplifier

The ground electrode was of the same kind as the recording electrodes In most animals recordings were taken from the left round window only

Stability of the preparation

During preliminary experiments it was found that changes of the position of the electrode relative to the membrane, too small to be visible under the dissecting microscope, caused gross changes in the size of the recorded cochlear microphonics The effects of such movements were abolished by drilling a small niche in the rim of the round window the electrode tip could be moved in this niche without concomitant changes in the size of the cochlear microphonics

In the experiments reported the animals were curarized and/or the electrode was placed in such a niche in the rim of the round window

Results

Effects on the action potential component of the round window response

The material presented in a preliminary report (FEX 1959) is included in the present series of experiments on 80 animals

It was found that electrical stimulation of the olivo-cochlear bundle in the floor of the fourth ventricle reduced or abolished the action potential component of the round window response to a click The effect was dependent upon the choice of stimulating parameters The optimal stimulus frequency for giving an effect was found to be of the order of 250—425 shocks/sec The same frequency range was found to be optimal for the inhibition of primary afferents as described above (p 40) With 2 V shocks given at the rate of 250 shocks/sec the inhibitory effect on the action potenial needed a few tenths of a second to be fully developed The same order of time was found for the decay of the effect following stimulation These time relations have been described by GALAMBOS (1956) and have not been studied in further detail here

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The results of GALAMBOS (1956) *suggest that the crossed olivo cochlear fibres* act somewhere in the region where acoustic energy is transformed into nerve impulses. The result of the many studies concerned with this transmission *mechanism (see* DAVIS *1961)* are used in the present work for a *further analysis* of the site of action of the system under study. The action of strychnine as reported by DESMEDT and MONACO (1959, 1960) as well as the results to be described here add further information as to how efferent endings may be functionally localized.

Technique and Procedure

Selection of experimental animals

The experiments in this series were carried out on 80 cats weighing between 1.5 and 3.5 kg. They were selected by the criteria described above (p. 8).

The preparation of the animal for the experiment

Most of the animals were decerebrated but some were kept under narcosis with Thiogenal. Stimulating electrodes were placed in the floor of the fourth ventricle and the bulla was exposed as described above. In several animals *the middle ear muscles were divided.*

Stimulating technique

The technique and the equipment used for electrical as well as for acoustical stimulation have been described above (pp. 10 and 11).

Recording technique

Chlorided silver wires with a diameter of 0.1—0.2 mm were used as electrodes: one electrode was placed on or near the round window and another was located a short distance away as a reference electrode. The wires were enclosed in thin polyethylene tubing and, for stabilization, the tubes and wires were knotted and embedded in dental cement on the rim of the bulla. The bulla was closed with dental cement in most of these animals.



Fig 21 Effect of tetanic electrical stimulation of the crossed efferent fibres on the cochlear microphonic at the round window A continuous tone has been presented in A and in B In B a tetanic burst has been triggered simultaneously with the sweep Note that there is an increase of the cochlear microphonic with a delay of 17—20 msec relative to the beginning of the burst.

Cat 26 10 59 anaesthetized with Thiogenal and curarized with Flaxedil Frequency of stimulating tone 1 500 c/s

The suppression of the action potential at the round window reportedly begins 20—30 msec after the first shock in a tetanic train (GALAMBOS 1956) Approximately the same time relationships were found in the present studies for the increase of the cochlear microphonic As shown in Fig 21, the increase

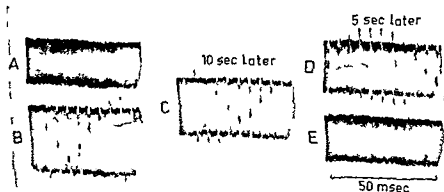


Fig 22 Effect of tetanic stimulation of the crossed efferent fibres on the cochlear microphon c at the round window A and E show the
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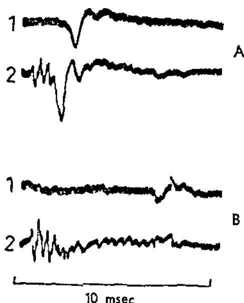


Fig 20 Effects of electrical tetanic stimulation of the crossed efferent fibres on click evoked responses at the round windows A shows the control response to a click at the left (1) and the right (2) round window In B an identical click is presented during prolonged tetanic stimulation *The action potential is* abolished in 1 and the cochlear *microphonic is increased in 2*

Cat 05 05 59 anaesthetized with Thiogenal and curarized with Flaxedil Loud speaker at right ear

Effects on the cochlear microphonic potential

The cochlear microphonic component of the round window response to sound was increased when tetanic stimulation was applied to the crossed olivocochlear bundles This effect became noticeable at approximately the same stimulus strength that produced a reduction of the action potential component of the round window response The minimal stimulus strength producing an effect corresponded to a stimulator output of 0 16 V With increasing stimulus strength the increase of the cochlear microphonic passed a maximum and at a stimulus strength 5—10 times threshold the increase changed to a decrease Since this change of sign of the effect on the cochlear microphonic was not seen in animals that were curarized with Flaxedil or in animals that had had the middle ear muscles cut, it was considered an effect of current spread to nerve fibres innervating the middle ear muscles

The increase of the cochlear microphonic with electrical stimulation of the olivo cochlear bundle was obtained when tested with clicks (Fig 20) as well as with continuous tones (Fig 21) It was apparent for tone frequencies from 500 c/s to 9 000 c/s Tones beyond this range were not investigated The well known phenomenon was identified that the cochlear microphonic grows with increasing sound pressure to reach a maximum after which the cochlear microphonic gets smaller with *further increase of* sound pressure (see e g Davis 1957) However at sound pressures giving maximal cochlear microphonic, tetanic stimulation of the crossed olivo-cochlear fibres increased the cochlear microphonic still further as it did at sound pressures both below and above this level

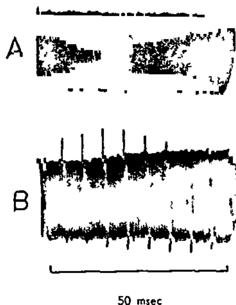


Fig 21 Effect of tetanic electrical stimulation of the crossed efferent fibres on the cochlear microphonic at the round window A continuous tone has been presented in A and in B In B a tetanic burst has been triggered simultaneously with the sweep Note that there is an increase of the cochlear microphonic with a delay of 17—20 msec relative to the beginning of the burst.

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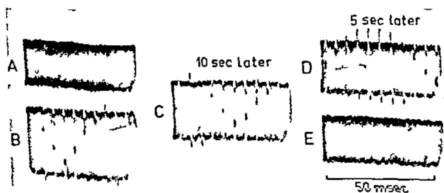


Fig 22 Effect of tetanic stimulation of the crossed efferent fibres on the cochlear microphonic at the *round window* A and E show the control responce In records B—D uninterrupted tetanic stimulation was applied to the floor of the fourth ventricle Note the decrease in augmentation of the cochlear microphonic from B—D Same cat and same stimulation parameters as in fig 21

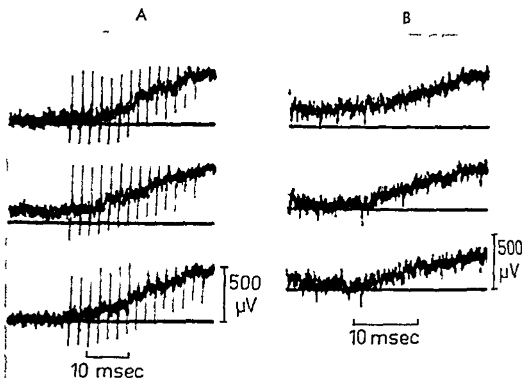


Fig 23 Effect of tetanic electrical stimulation of the crossed efferent fibres on the resting potential at the round window in two different experiments In A tetanic bursts were triggered with a delay relative to the sweep as is evident from the shock artifacts in B the tetanic bursts and the sweeps were triggered simultaneously (shock artifacts not clearly visible) In A is seen how the resting potential begins to increase after a latency of the order of 7 msec In B this latency is of the order of 12 msec Decerebrate cat with middle ear muscles cut curarized with Flaxedil Calomel electrodes Cat 29 05 61 (A) Decerebrate cat curarized with Flaxedil Ag—AgCl electrodes Cat 07 02 61 (B)

of the cochlear microphonic became evident 17—20 msec after the beginning of the tetanic burst

In many experiments it was noted that the increase in cochlear microphonic tended to diminish with prolonged tetanic stimulation, as shown in Fig 22 The time relationships of this phenomenon have, however not been analyzed in this study

The increase of the cochlear microphonic was maximally of the order of 3 db (Fig 22 B) and was dependent on the parameters of the electrical stimulation in that it increased with increasing stimulus strength from threshold strength up to levels 5—10 times threshold The optimal stimulus frequency for effects on the cochlear microphonic the action potential and the resting potential at the round window (see below p 49) was found to be 250—425 shocks/sec

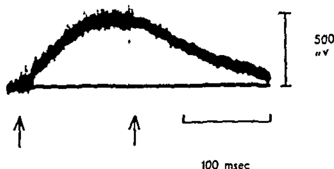


Fig 24 In this figure is shown the effect of tetanic electrical stimulation of the crossed efferent fibres on the resting potential at the round window A tetanic burst has been triggered with a short delay relative to the sweep The arrows mark the beginning and the end of the burst. Note that the increase of the resting potential has reached a plateau within 0 1 sec and that the prestimulatory level has not been reached within 0 15 sec after cessation of stimulation Same cat as in fig 23 A same stimulation parameters

Effects on the resting potential at the round window

The endocochlear resting potential was discovered and described by von BEKESY (1950 1952 *a*) in the guinea pig He found an endolymphatic resting potential of + 80 mV relative to the perilymph of the tympanic scale, von BEKÉSY also demonstrated that as a reflection of the endolymphatic potential there was a potential of about + 2 mV at the round window membrane relative to a reference electrode located near the wall of the bulla

In 3 cats the resting potential at the round window was found to be 6 0—7 5 mV relative to a reference point outside the cochlea This potential increased in response to electrical stimulation of the crossed olivo-cochlear bundle It was difficult to define the beginning of the effect as seen in Fig 23 The estimated latencies in 6 cats have ranged between 7 and 13 msec

The effect of electrical stimulation of the crossed olivo-cochlear bundle on the resting potential needed some time to reach its maximal value just as the effect on the cochlear microphonic the action potential and the primary auditory afferents The times of rise and decay are illustrated in Fig 24 Using certain stimulating parameters the increase in the resting potential at the round window to electrical stimulation was only transient This is exemplified by Fig 25 where the maximal effect is reached within 0 2 sec. The resting potential returned almost to its original level in spite of continued stimulation of the crossed olivo-cochlear fibres With cessation of stimulation, there seemed to be a small overshoot to a value below the original resting potential

The change of resting potential was maximally 0 5 mV It depended on

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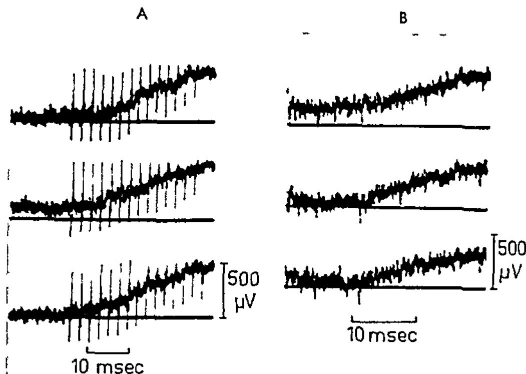


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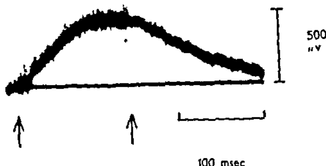


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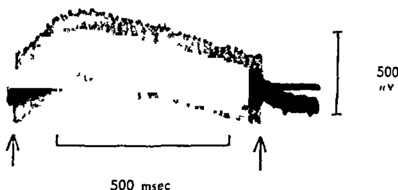


Fig 25 In this figure is shown the effect of tetanic electrical stimulation of the crossed efferents on the resting potential at the round window A tetanic burst has been triggered with a short delay relative to the beginning of the sweep The arrows mark the beginning and the end of the burst Note that the resting potential returned almost to its original value during continued stimulation of the crossed efferents With cessation of stimulation there was a slight overshoot of the resting potential Same cat as in fig 23 A and fig 24, same parameters of stimulation, expect for duration of burst

stimulus strength and frequency as did the other effects of crossed olivo-cochlear stimulation

As a control procedure a shallow short cut was placed 3 mm lateral of the stimulating electrodes in the midline, between these and the recording electrode (in 5 animals) After such a cut electrical stimulation no longer produced any changes of the action potential, cochlear microphonic or resting potential at the round window in animals under Flexedil and/or the middle ear muscles divided (*cf* GALAMBOS 1956)

The action of strychnine on the response to stimulation of the crossed olivo cochlear fibres

DESMEDT and MONACO (1960) showed that intravenous injection of strychnine sulphate in doses of 0 05—0 15 mg/kg body weight blocked the effect of electrical stimulation of the olivo cochlear bundle on the action potential at the round window Strychnine sulphate applied locally to the one cochlea blocked the ipsilateral response while inhibition of the action potential could still be seen in the other ear The augmentation of the cochlear microphonic (FEX 1959) could also be blocked by strychnine (DESMEDT and MONACO 1961)

In the present series of experiments strychnine sulphate given intravenously in doses of 0 1—0 15 mg/kg body weight was found to block the effect of olivo cochlear bundle stimulation on the action potential, cochlear micro phonic and resting potential at the round window A strychnine block that seemed total at one stimulus strength could appear as a partial block at

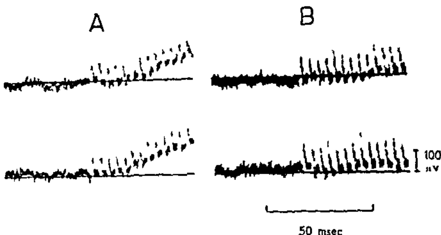


Fig 26 The blocking effect of strychnine on the increase of the resting potential at the round window from efferent stimulation Tetanic bursts were triggered with a delay relative to the beginning of the sweeps. In A the result of efferent stimulation is seen before the administration of strychnine Between A and B strychnine was given intra venously 130 µg/kg body wight. Decerebrate cat with middle ear muscles cut, curarized with Flaxed I Cat 28 05 61

higher stimulus strengths In Figs 26 and 27 the change of resting potential in response to electrical stimulation before and during the strychnine block, is illustrated In Fig 27 A the maximal effect of the electrical stimulation was reached within 0 2 sec while during the strychnine block in B the resting potential was still increasing at the end of the 0 25 sec tetanic burst and the change of resting potential at that point was only half of that of Fig 27 A

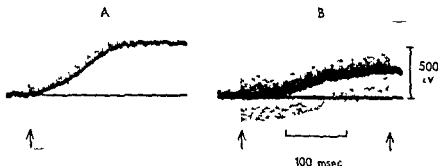


Fig 27 The blocking effect of strychnine on the resting potential at the round window from efferent stimulation Tetanic bursts have been triggered with a delay relative to the beginning of the sweeps The arrows mark the beginning and the end of the burst. In A the result of efferent stimulation is seen before the administration of strychnine Between A and B strychnine has been given intravenously 130 µg/kg body weight. Same animal and same data as in fig 26

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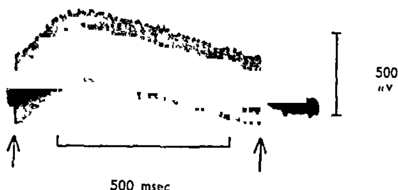


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stimulus strength and frequency as did the other effects of crossed olivo-cochlear stimulation

As a control procedure a shallow short cut was placed 3 mm lateral of the stimulating electrodes in the midline, between these and the recording electrode (in 5 animals) After such a cut electrical stimulation no longer produced any changes of the action potential, cochlear microphonic or resting potential at the round window in animals under Flexedil and/or the middle ear muscles divided (*cf* GALAMBOS 1956)

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In the present series of experiments strychnine sulphate given intravenously in doses of 0 1—0 15 mg/kg body weight was found to block the effect of olivo-cochlear bundle stimulation on the action potential, cochlear micro phonic and resting potential at the round window A strychnine block that seemed total at one stimulus strength could appear as a partial block at

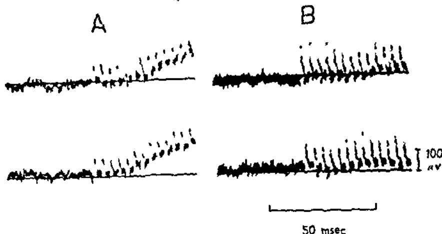


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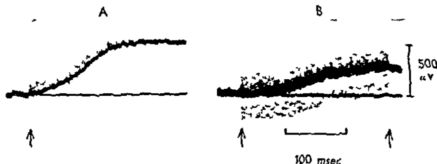


Fig 27 The blocking effect of strychnine on the *resting potential* at the round window from efferent stimulation. Tetanic bursts have been *triggered with a delay* relative to the beginning of the sweeps. The arrows mark the beginning and the end of the burst. In A the result of efferent stimulation *is seen before* the administration of strychnine Between A and B strychnine has been given *intravenously*, 130 μg/kg body weight Same animal and same data as in fig 26

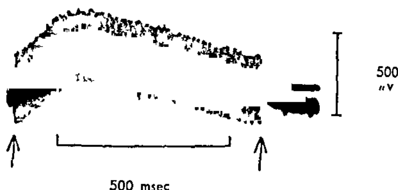


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In the present series of experiments strychnine sulphate given intravenously in doses of 0 1—0 15 mg/kg body weight was found to block the effect of olivo-cochlear bundle stimulation on the action potential, cochlear microphonic and resting potential at the round window A strychnine block that seemed total at one stimulus strength could appear as a partial block at

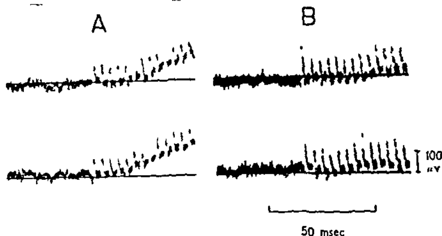


Fig 26 The blocking effect of strychnine on the increase of the resting potential at the round window from efferent stimulation Tetanic bursts were triggered with a delay relative to the beginning of the sweeps In A the result of efferent stimulation is seen before the administration of strychnine Between A and B strychnine was given intravenously 130 μg/kg body wight Decerebrate cat with middle ear muscles cut, curarized with Flaxed l Cat 28 05 61

higher stimulus strengths In Figs 26 and 27 the change of resting potential in response to electrical stimulation before and during the strychnine block, is illustrated In Fig 27 A the maximal effect of the electrical stimulation was reached within 0 2 sec while during the strychnine block in B the resting potential was still increasing at the end of the 0 25 sec tetanic burst and the change of resting potential at that point was only half of that of Fig 27 A.

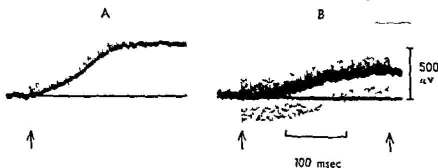


Fig 27 The blocking effect of strychnine on the resting potential at the round window from efferent stimulation Tetanic bursts have been triggered with a delay relative to the beginning of the sweeps The *arrows mark* the beginning and the end of the burst In A the result of efferent stimulation is *seen before* the administration of strychnine Between A and B *strychnine has* been given intravenously 130 μg/kg body weight Same animal and same data as in fig 26

4**

DISCUSSION

Basic assumptions

Before the results can be discussed the validity of the basic assumptions underlying the study must be submitted to analysis. These assumptions are that Chapter I deals with crossed olivo-cochlear fibres (see below, A) and that the effects described in Chapters II and III are due to electrical stimulation of crossed olivo-cochlear fibres and of no other fibre systems (see below, B). The experimental situation when the cochlear nerve was stimulated close to the recording electrode in the vestibulo-cochlear anastomosis will also be considered (see below, C).

It has been regarded a necessary and adequate criterion for the identification of a crossed efferent fibre in the vestibulo-cochlear anastomosis, that it could be electrically driven from the floor of the fourth ventricle. The possibility that fibre groups other than the crossed olivo-cochlear ones have been co-stimulated should now be considered in the light of pertinent data on current spread and histological findings.

GALAMBOS (1956) found that if the stimulating electrodes in the floor of the fourth ventricle were moved in the midline more than 2—3 mm from an optimal position, the inhibitory effect on the action potential at the round window was completely lost. This finding has been confirmed in the present work, and several times it has been found that when the stimulating electrodes were moved 1—2 mm in the midline the stimulus strength had to be increased by a factor of five to ten in order to give the effect obtained at the original position. When a shallow cut was placed 3 mm lateral to the stimulus site in the midline, the electrical stimulation no longer produced any changes at the ipsilateral round window in animals deprived of funtion on the part of the middle ear muscles. Spread to facial fibres innervating the stapedius muscle appeared at stimulus strengths 5—10 times threshold. The facial genua are closer to the stimulating electrodes than any other structures that need be considered.

A. Fibres in the vestibulo-cochlear anastomosis may be 1) unmyelinated autonomic fibres [such fibres have not been found in the anastomosis but have not either been finally excluded (GACEK and RASMUSSEN 1961)], 2) primary auditory afferent fibres (RASMUSSEN 1953*a*), 3) uncrossed olivo-cochlear fibres (RASMUSSEN 1960) and 4) crossed olivo-cochlear fibres RASMUSSEN 1946). The question now arises if any fibres other than group 4 possibly might be stimulated in the floor of the fourth ventricle.

ad 1 LORENTE de No (1937) mentions fibres innervating blood vessels in the cochlea of young rats and claims that these fibres reach the inner ear through an anastomosis between the facial and the cochlear nerve, RASMUSSEN (1946) and PORTMANN (1952) both believe that LORENTE de No actually had seen the vestibulo cochlear anastomosis itself There is thus no evidence for his hypothetical autonomic fibres to the region stimulated here, as defind in previous paragraph Neither RASMUSSEN nor PORTMANN has recognized any autonomic fibres in the vestibulo-cochlear anastomosis Even if such fibres exist it is most unlikely that they could be recorded from by the techniques used in the present work

ad 2 After destruction of the spiral ganglion a central projection of degenerated primary neurones has been found in the trapezoid body (LEWY and KOBRAK 1936) and the dorsal cochlear nucleus (STOTLER 1961) which are far from the site of the electrodes It is therefore concluded that in the present work primary auditory afferent fibres cannot have been antidromically stimulated by spread of current

ad 3 Little has been published on the uncrossed olivo-cochlear fibres According to RASMUSSEN (1960 *cf* his Fig 84) these fibres run from the S-shaped olivary nucleus in a dorso-lateral direction and join the crossed olivo-cochlear fibres from the opposite side lateral to the facial root These structures are also outside the region that can be reached by current spread By the electrical midline stimulation uncrossed olivo-cochlear fibres may yet have been activated through unknown internuncial neurones or through the pathway of crossed olivo cochlear fibres — cochlear nucleus (RASMUSSEN 1960) — superior olive (STOTLER 1953) — uncrossed olivo cochlear neurones but this possibility seems to be highly speculative, even though it cannot be excluded

ad 4 Of the different possibilities listed there remains only the crossed efferent bundle In histological sections from five animals electrode tracts were found in or close to, crossing bundles that satisfactorily fitted the description given by RASMUSSEN (1946) for the crossed olivo-cochlear fibres

B Concerning the effects described in Chapters II and III fibres other than the ones running in the vestibulo cochlear anastomosis have to be considered The possibilities are 1) autonomic fibres, 2) primary cochlear afferents 3) uncrossed efferent cochlear fibres 4) crossed efferent cochlear fibres 5) fibres innervating the middle ear muscles

ad 1 No parasympathetic fibres have ever been shown to travel with the eighth nerve in the modiolus (*cf* SMITH 1961) Investigators utilizing silver stains (LORENTE de No 1937, SMITH 1951) have not been able to demonstrate any sympathetic fibres accompanying blood vessels peripheral to the modiolus

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C The apical part of the cochlear nerve was stimulated electrically and evoked fibre activity recorded in crossed olivo-cochlear fibres in the vestibulo-cochlear anastomosis half a mm from the site of stimulation The crossed olivo-cochlear fibres could have been activated through 1) current spread from the stimulating electrode, 2) an artificial synapse (GRANIT and SKOGLUND 1945 *a* and *b*) or some kind of ephaptic transmission (*cf* LLOYD 1942) between primary auditory fibres and crossed efferent fibres, 3) physiological connexions between auditory afferents and the cells of origin of the crossed olivo-cochlear fibres

ad 1 Some of the evoked potentials had latencies below 1 5 msec and were therefore attributed to current spread In the other group of potentials the latencies (3 5—10 msec) were too long to be explained in this manner

ad 2 The latencies 3 5—10 0 msec of the responses under discussion also presuppose that the site of abnormal transmission must be placed far out in the periphery where postulated lesions in adjoining afferent and *efferent fibres* necessarily would be sparse GRANIT and SKOGLUND (1945 *a* and *b*) found fresh sections and low temperature favourable for transmission through artificial synapses Lesions of afferent and efferent fibres caused by drilling and pulling must have been irregular and were *fresh only* during the beginning of the procedures The temperature of the room was not below 20° C

ad 3 According to RASMUSSEN (1960) the cells of origin of the crossed efferent fibres receive their afferent connexions predominantly from the contralateral cochlear nuclei The cochlear nerve that was electrically stimulated was thus probably the main pathway *for activating the crossed olivo-cochlear* neurones under physiological conditions Whether or not electrical stimulation of the cochlear nerve is a more effective stimulus for the crossed olivo-cochlear *fibres* than a physiological one is not known

The latencies *for the first* impulses in crossed efferent fibres initiated by electrical stimulation of the cochlear nerve were found to be 3 5—10 0 msec GALAMBOS et al (1959) have reported latencies to sound of 2 5—10 0 *msec in the* superior olive The present data are thus compatible with *earlier* findings

It is therefore concluded *that in the present* experiments the crossed olivo-cochlear fibres were activated through *their cells* of origin when the cochlear nerve was electrically stimulated

Earlier observations on efferent endings

ENGSTRÖM (1958) utilizing electron microscopic techniques demonstrated in the guinea pig cochlea that there was a *marked difference* between two kinds of nerve ending In particular the *first row* of outer hair cells was found to be

Therefore it seems improbable that the effects studied have been due to activation of autonomic fibres running to the organ of Corti

Autonomic fibres to other parts of the cochlea might change inner ear activity by altering blood supply or as a consequence of secretory processes In the work of KONISHI, BUTLER and FERNÁNDEZ (1961) will be found the shortest time ever reported for decrease of cochlear potentials (in guinea pig) through interference with the blood supply to the cochlea After interruption of the arterial blood supply to the cochlea by sectioning the anterior inferior cerebellar artery or by abrupt occlusion, the cochlear potentials started to decrease with a latency of 2—3 sec The endolymphatic resting potential, the summating potential, the action potential and the cochlear microphonic were studied in that work The data permit the conclusion that no changes occur during the first second of arterial occlusion

SMITH (1957), in an electron microscopic study of the structure of the stria vascularis reported findings suggestive of a secretory function in this organ Experiences with the sublingual glands may be pertinent for a discussion of whether such a function might be of importance in this connexion (LUNDBERG 1958, also for references) When fibres to the sublingual gland were stimulated secretory potentials were recorded from the gland with latencies of the order of 1 sec and further experimental evidence strongly supported the assumption that these secretory potentials depended upon active ionic transport directly related to secretion

A critical review of earlier findings of changes of cochlear microphonics induced by stimulation of the sympathetic system is given by BEICKERT, GISSELSSON and LOFSTROM (1956) together with results of their own experiments on cats They found changes that needed minutes to become maximal which could not be produced in animals with the middle ear muscles out of function

Therefore the participation of autonomic nerve fibres in the production of the early changes described in Chapters *II* and *III* can be excluded

ad 2 and 3 It was concluded above (see A 2) that no primary auditory neurones were within reach of the stimuli applied to the floor of the fourth ventricle This also holds (see above, *A 3*) *for the uncrossed olivo-cochlear* fibres

ad 4 and 5 The effects under discussion were seen in animals in which the middle ear muscles were *put out of function* and so could not be due to the action of these muscles

In A 4 it was inferred that the crossed olivo cochlear fibres actually were the ones electrically *stimulated from the floor* of the fourth ventricle and it is now concluded that such stimulation alone produced the effects discussed in Chapter II and Chapter III

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provided with a very large system of nerve terminals filled with rounded granulas. The inference was that these nerve endings actually represented efferent fibres although it seemed surprising that they occured in such quantities as to presuppose a very considerable amount of terminal splitting. The presence of the two kinds of nerve ending was confirmed by SPOENDLIN (1960) in cats and by IURATO (1961) in rats.

ENGSTROM and FERNÁNDEZ (ENGSTROM 1961) in a discussion have reported observations to the effect that after sectioning the efferent bundle in the fourth ventricle the number of granulated nerve endings of the outer hair cells was diminished. Whether only the crossed efferent bundle or both the crossed and uncrossed ones were sectioned is not apparent. SMITH (1961) found nerves of small calibre winding themselves around the radial nerve fibres — held to be afferent — and apparently in synaptic contact with them. They sometimes also touch the hair cells. It was *suggested that these nerves of small calibre* are identical with RASMUSSEN's efferent bundle.

Histological findings thus clearly demonstrate a double innervation of the hair cell region and the studies of the effect of lesions in the fourth ventricle has demonstrated that one of these systems of terminals is efferent. However it must again be stressed that as yet there has been no differentiation between the two efferent systems.

GISSELSSON (1950) found evidence of acetylcholinesterase in the endolymph of cat and CHURCHILL and SCHUKNECHT (1956) could localize this enzyme to the hair cell region of the organ of Corti and to nerve fibres orientated towards this organ. The acetylcholinesterase in the organ of Corti diminished or disappeared *after lesions in the floor of the fourth* ventricle (CHURCHILL and SCHUKNECHT 1959, SCHUKNECHT, CHURCHILL and BORAN 1959). They inferred that its disappearance was due to section of the crossed efferent fibres but SCHUKNECHT (1960) added: "I was fortunate in that I made my incisions for cutting the olivocochlear bundle in the sulcus limitans far enough laterally to *get the homolateral branch which Dr. Rasmussen* described yesterday and which I had not known about before."

Further studies on the acetylcholinesterase content of the organ of Corti were made in cats and guinea pigs by VINNIKOV and TITOVA (1958) who showed that one hour of stimulation with loud tones caused a decrease in the concentration of acetylcholinesterase. This fall was localized to the upper part of the cochlea when low frequency tones were used and in the lower part after high frequency tone stimulation. *DEL BO and CONTI (1961)* found that in guinea pigs younger than ten days acetylcholinesterase was present only in the basal part of the organ of Corti.

A further extension of the possibilities of this technique was made by WER

SALL, HILDING and LUNDQUIST (1961) who completed an electron microscopic study of the distribution of acetylcholinesterase in the organ of Corti in the guinea pig. They stated that it proved impossible to localize the enzyme to the one or the other type of nerve ending because of artefacts from shrinking of the preparations, but in general the distribution of it corresponded to that of the large nerve endings as described by ENGSTROM (1958).

A clue to a differentiation between the two efferent systems may be had from the findings of DESMEDT and MONACO (1960, 1961) who demonstrated that strychnine blocked the inhibition of the action potential as well as the augmentation of the cochlear microphonic at the round window, as caused by electrical stimulation of the crossed efferent fibres. Since there has been no reports of strychnine blocking the action of cholinergic fibres in mammals, the findings of DESMEDT and MONACO suggest that the crossed efferent fibres are non cholinergic. It would follow that the acetylcholinesterase is part of the uncrossed efferent system. This hypothesis may be tested experimentally by techniques of the kind used in this study.

The site of action of the crossed cochlear efferents

In Chapter III it has been demonstrated that the resting potential, the cochlear microphonic and the action potential at the round window all are affected by electrical stimulation of the crossed efferent fibres.

The resting potential at the round window is a reflection of the endolymphatic resting potential (von BÉKÉSY 1952*a*) which originates in the stria vascularis (DAVIS, DEATHERAGE, ROSENBLUT, FERNÁNDEZ, KIMURA and SMITH 1958, MISRAHY, DE JONGE, SHINABARGER and ARNOLD 1958, TASAKI and SPYROPOULOS 1959). The site of the cochlear microphonic of the inner ear was placed by von BÉKÉSY (1952*b*) to the organ of Corti. TASAKI, DAVIS and ELDREDGE (1954) using the technique of von BÉKÉSY claimed that the hair cells are the source of the microphonic potential. Synchronous firing of a large number of nerve impulses is the accepted explanation of the action potential at the round window (see DAVIS 1957).

Present concepts of the mechanism by which acoustical energy is transduced into nerve impulses are summarized by DAVIS (1961) in the following words: "The stria vascularis of the cochlea is such a 'battery' (fig. 7) and it hyperpolarizes the hair cells. The receptor potentials of the ear are the cochlear microphonic and the summating potential. Both potentials derive energy from the hair cells and are controlled in the hair cells by a mechanical action, but they also draw additional energy from the stria vascularis."

It has been argued above that the stria vascularis as a secretory organ cannot cause the changes under discussion. The observed increase of the

resting potential at the round window is then probably due to a decreased impedance of the organ of Corti to the current driven by the endocochlear potential

It was shown above that the cochlear microphonic is increased by the efferent stimulation while the action potential at the round window is decreased From the study of primary auditory afferents it was concluded that no facilitatory processes were concealed behind the decrease

Present knowledge concerning the relations of the resting potential, the cochlear microphonic and the action potential at the round window permit no conclusion as to whether or not the observed changes of these potentials actually are causally related It is impossible at this stage to conclude anything more definite concerning which structures in the organ of Corti are affected by the efferent activity In general however, the present findings are fully compatible with the histological suggestions according to which the crossed efferents terminate on hair cells

There seems to be a discrepancy between the effects of efferent stimulation on the receptor potential and on the action potential inasmuch as the former increases the latter diminishes Further studies of the effects of efferent stimulation on all the cochlear potentials, including the summation potential and on the primary afferents may solve the discrepancy observed Such studies may also contribute to a deeper understanding of the mechanisms whereby sound is translated into nerve impulses

Activity and function of the crossed cochlear efferents

Although the crossed olivo cochlear neurones were adequately activated by the cochlear nerves their firing pattern did not closely follow that of the primary and secondary auditory neurones The differences in firing pattern and the long latencies indicate that the input to the crossed olivo cochlear neurones would be integrated over a considerable time During these integrative processes irregularities in the input activity are smoothed out

A reproducible threshold of sound pressure at a best frequency and a well defined band width of tone frequency at different pressure levels were some of the features common to afferents and efferents

Crossed olivo cochlear fibres in the basal fascicle of the vestibulo cochlear anastomosis generally responded to higher tone frequencies than did the fibres in the apical fascicle (p 25) This leads to the conclusion that sound of high tone frequencies to one ear suppresses the response to high frequency sound in the other ear It may well be that this is an expression of a general principle according to which the nerve endings of the crossed efferent fibres are orderly arranged in a spatial sequence similar to that of the afferent nerve endings in

the cochlea so that afferents and efferents from homotopic cochlear points in opposite cochleas are connected The selective depletion of acetylcholinesterase — in the basal part of the cochlea after stimulation with high tone frequencies, in the apical part after low tones (VINNIKOV and TITOVA 1958) — is also suggestive of such an orderly arrangement of efferent nerve endings, presumably those of the uncrossed efferent system (see above for discussion, p 56)

For the understanding of the function of the crossed efferent system it should be kept in mind that the superior olive is an auditory relay on to which afferent auditory neurones converge most of which are second order neurones (STOTLER 1953) ROSENZWEIG and AMON (1955) have found binaural interaction of clicks in the superior olive, as studied with macroelectrodes in acute experiments on cats

The experiments reported here were not ideal for analyzing this bilateral representation since the ear providing the predominant afferent inflow (*cf* RASMUSSEN 1960) to the crossed efferents under study had been put out of function as part of the preparation for the experiment In spite of these experimental shortcomings it could be demonstrated that sound stimulation of the ear ipsilateral to the superior olive increased the responsiveness of these neurones to electrical stimulation of the contralateral auditory nerve This result seems to be in harmony with those of GALAMBOS *et al* (1959) although the experimental conditions differed They suggested further that nucleus accessorius contains a class of neurones concerned chiefly with preserving the exact time of arrival of stimuli at the cochlea This is of interest because dorsal cells in the region of the rostral third of nucleus accessorius give origin to the crossed efferent cochlear fibres (GALAMBOS *et al* 1959)

It appears from the results of Chapters I and II and the foregoing discussion on the significance of electrical stimulation of the cochlear nerve that the crossed efferents form part of a closed feedback loop (*inner ear — cochlear nucleus — contralateral superior olive — crossed efferents back to ear*)

The results of Chapters II and III may represent sub-maximal effects since they were derived from experiments with the proposed feedback loop closed, with one exception cat 08 12 61 in which the crossed efferents were cut above the stimulating electrode in the fourth ventricle.

It might be of interest to compare the crossed cochlear feedback system with that involving the middle ear muscles It is well known that the reflex muscle contraction to sound is largely (*e g* in cat, *cf* SIMMONS 1959), or exclusively (in man, *cf* KLOCKHOFF 1961), due to the stapedius The reflex arc of this muscle is not exactly known but probably runs in auditory afferents to the superior olive, then to the facial nucleus and further on to fibres

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A reproducible threshold of sound pressure at a best frequency and a well defined band width of tone frequency at different pressure levels were some of the features common to afferents and efferents

Crossed olivo cochlear fibres in the basal fascicle of the vestibulo cochlear anastomosis generally responded *to higher tone frequencies* than did the fibres in the apical fascicle (p 25) This leads to the conclusion that sound of high tone frequencies to one ear suppresses the response to high frequency sound in the other ear It may well be that this is an expression of a general principle according to which the nerve endings of the crossed efferent fibres are orderly arranged in a spatial sequence similar to that of the afferent nerve endings in

destined for the stapedius (*cf* JEPSEN 1955) It has been shown by MÖLLER (1961) by impedance measurements on man that the stapedius reflex nearly always was more sensitive to ipsilateral stimulation The sensitivity was defined by him as the intensity required for a definite percentage of the maximum impedance change

Latencies from first cochlear potentials to the earliest muscle potentials were for the stapedius 6 ± 0 5 msec and for the tensor tympani 7 ± 0 7 msec in decerebrated cats (ELIASSON and GISSELSSON 1955) WERSÄLL (1958) found in rabbits under pentobarbitone that the attainment of 50 per cent of the final reflex tension was reached for the stapedius muscle in 40—151 msec and for the tensor tympanic muscle in 44—174 msec. This time depended but little on sound intensity SIMMONS (1959) who monitored round window activity through chronically implanted electrodes in awake cats reported attenuation of up to 30 db of the cochlear microphonic through the action of the middle ear muscles

Comparing available figures *i e* those given now for latency, course of development and attenuation of afferent input by the reflex route and corresponding ones presented above for stimulation by the efferent fibres it is of some interest to note that they are of the same general order

Recent experimental results (KLOCKHOFF and ANDERSON 1960, KLOCKHOFF 1961 SIMMONS GALAMBOS and RUPERT 1959 HUGELIN, DUMONT and PAILLAS 1960) show that the control of the middle ear muscles is not directed according to a rigid pattern through a simple feedback loop but that setting mechanisms may to a large extent regulate their activity

Recently several experiments besides those already referred to have been performed in an effort to trace centrifugal effects on auditory activity at the level of the inner ear Decrease of the action potential component at the round window as an expression of habituation has been found in cats with chronically implanted electrodes (ALTMAN 1960) as well as in relation to the development of an orientation reaction (MARUSEVA 1961) The findings were also obtained in cats in which the middle ear muscles had been cut and so were interpreted as true centrifugal effects GALAMBOS (1960) reported a series of negative findings in experiments of a similar type

GALAMBOS (1960) also failed to find evidence of crossed olivo-cochlear activation when shocks were applied to the region of the brachium of the inferior colliculus These findings are most interesting because RASMUSSEN (1953*b* 1955) has reported descending degeneration following lesions of the inferior colliculus and/or the dorsal nucleus of the lateral lemniscus This degeneration projects predominantly to that region of the superior olive which previously was found to be a site of origin of the crossed olivo-cochlear fibres

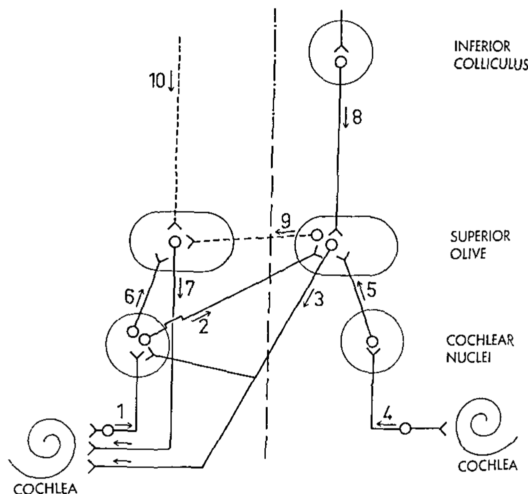


Fig 28 *Diagram illustrating the feedback system of the left cochlea* *Only primary* afferents shown for the right cochlea, although every connexion depicted has its contralateral homotopic counterpart The terms "ipsilateral" and "contralateral" refer to the left ear 1, primary auditory afferents from cochlea to ipsilateral cochlear nuclei, 2, connexions from ipsilateral cochlear nuclei crossing to the contralateral superior olive, 3, the efferent tract of olivo-cochlear fibres from the contralateral superior olive crossing to the cochlea — Connexions 1—2—3 constitute the closed feedback loop 4, primary auditory fibres from the contralateral cochlea to the contralateral cochlear nuclei, 5, connexions from the contralateral cochlear nuclei to the contralateral superior olive Through connexions 4—5 the closed feedback loop of connexions 1—2—3 can be influenced — 6, connexion from the ipsilateral cochlear nuclei to the ipsilateral superior olive, 7, the uncrossed efferent connexion from the ipsilateral superior olive to the cochlea Connexions 1—6—7 may constitute a closed feedback loop — 8, connexions from the contralateral inferior colliculus to the contralateral superior olive *Tract 8 may* influence activity in the closed feedback loop of connexions 1—2—3 — 9, proposed connexions from the contralateral superior olive to the uncrossed efferent neurones, which would make it possible for input to the contralateral ear to influence activity in the proposed closed feedback loop of connexions 1—6—7 No direct connexions between the contralateral cochlear nuclei and the ipsilateral uncrossed efferent neurones are known — 10, proposed connexions from nuclei central to the superior olive to the uncrossed efferent neurones, which would enable central control of the proposed feed back loop of connexions 1—6—7

SUMMARY

In the first part of this study a technique has been developed for recording from single crossed olivo-cochlear efferent fibres in the vestibular-cochlear anastomosis, decerebrate cats have been used in which the auditory function of the left ear has been destroyed as part of the preparation

Crossed olivo-cochlear fibres could be activated by sound applied to the contralateral ear The activity in the neurones has been analyzed with respect to resting activity, firing pattern, 'best frequency', tone frequency, band width, and latency

The crossed olivo cochlear fibres responded as a rule with a low, regular firing rate without initial bursts This indicated that the input to the crossed efferents would be integrated over a considerable time

Crossed olivo-cochlear fibres in the basal fascicle of the vestibulo-cochlear anastomosis generally responded to higher tone frequencies presented to the contralateral ear than did the fibres in the apical fascicle This may be an expression of a general principle according to which afferents and efferents from homotopic cochlear points in opposite cochleas are connected

Crossed efferent fibres were also activated by electrical stimulation of the ipsilateral auditory nerve This activity was analyzed with respect to latency and firing pattern

Crossed efferents in the cochlea showed increased responsiveness to electrical stimulation of the ipsilateral auditory nerve when sound was presented to the contralateral ear

In the second part of the study a technique has been described for recording from single primary auditory afferents Decerebrate cats with both cochleas intact were used A histological criterion for the identification of primary auditory neurones has been introduced The effect of electrical stimulation of the crossed efferents on primary auditory neurones was studied Such stimulation inhibited resting activity as well as sound evoked activity in the primary auditory neurones Neither facilitation nor rebound after inhibition was seen.

In the third part of the study the effect of electrical stimulation on cochlear potentials was studied with macroelectrodes at the round window Such stimulation increased the resting potential, increased the cochlear microphonic (seen earlier) and diminished the action potential (GALAMBOS 1956) It was concluded that the effect on the resting potential was due to a decreased impedance of the organ of Corti to the current driven by the endocochlear potential

DESMEDT (1960, foot note p 155) however, found it possible to record a decrease of the evoked action potentials both at the round window and in the cochlear nucleus when curarized cats were stimulated in this region It is not known if the effect at the round window was due to the crossed or the uncrossed bundle, but the data favour the former alternative

The present study has thus given the following main new results Efferent fibres in the cochlea from the contralateral superior olive are activated by the *afferent fibres from the same cochlea They thus form part of a feedback* loop within the auditory system The activity in this loop is influenced by input from the contralateral ear The action of the crossed efferents on the primary afferents is purley inhibitory and takes place in the organ of Corti simultaneously with an increase of the resting potential and with an increase of the cochlear microphonic at the round window

In Fig 28 the feedback control of the auditory input has been sketched for one side A closed loop is formed by primary auditory afferents (1) from the cochlea secondary auditory fibres (2) crossing to the contralateral *superior* olive and the crossed olivo cochlear fibres (3) returning to the cochlea The feedback control of the auditory input through this loop is influenced by afferents (4—5) from the contralateral ear to the contralateral superior olive The two symmetrical closed loops controlling each ear (only one side sketched) are *thus interconnected and, very probably, together with* the uncrossed olivo cochlear efferents (7) form a complex feedback control mechanism This system is very likely regulated from higher centres The connexion (8) between the region of the inferior colliculus and the cells of origin of the *crossed* cochlear efferents may be one of the possible pathways for such a centrifugal control

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The results have been further discussed in the light of current knowledge of the double innervation of the organ of Corti

The results permit the conclusion that auditory afferent fibres and the crossed efferent fibres together form a closed feedback loop within the auditory system The efferents of this loop are also activated from the contralateral ear These main new results lead to the final conclusion that the two crossed olivo cochlear bundles are part of a complex feedback control mechanism

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STUDIES ON THE ANTABUSE — ALCOHOL REACTION IN RABBITS

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INTRODUCTION

It was already known at the beginning of this century that certain substances affect the human organism in such a way that intake of even very small amounts of alcohol produces characteristic, extremely unpleasant symptoms. However, no therapeutic application of this phenomenon was made until JACOBSEN and co-workers in 1945 discovered that alcohol elicits a similar reaction in human subjects pretreated with *tetraethylthiuramdisulfide* (disulfiram). They performed a series of studies on the effect of this substance alone and in combination with alcohol, and these studies led to its introduction in the treatment of alcoholism under the name of Antabuse.

The syndrome which is elicited by alcohol in subjects pretreated with Antabuse is usually called *the Antabuse-alcohol reaction*, and circulatory and respiratory manifestations are prominent in its clinical picture. A similarly altered response to small amounts of alcohol has apparently never previously been convincingly demonstrated in animals pretreated with Antabuse in spite of several attempts. This has made the experimental analysis of the Antabuse-alcohol reaction difficult, and certain important aspects of its mechanism are still to a large extent obscure. This particularly holds for the circulatory changes during the reaction and for the exact role of acetaldehyde, the chief primary breakdown product of alcohol.

The present investigation was prompted by the observation that a small alcohol dose, which never appreciably affected normal rabbits, produced marked circulatory and respiratory effects in a rabbit pretreated with Antabuse. As these effects appeared to be similar to those seen during the human Antabuse-alcohol reaction a further study was undertaken.

cided roughly with the rise and fall in blood acetaldehyde (HINE *et al* 1952), and ASMUSSEN, HALD and LARSEN (1948 b) were able to reproduce several of these manifestations by infusing acetaldehyde into normal human subjects not pretreated with TETD Other findings indicated that acetaldehyde was probably not an intermediate product in normal metabolism (without ethanol) (JACOBSEN 1950) It was concluded from these findings that the TETD-ethanol reaction in man was due to accumulation of acetaldehyde in the blood

An effect of TETD on acetaldehyde metabolism has been shown in a number of further studies on laboratory animals Increases in the concentration of acetaldehyde in the blood after TETD + ethanol were reported in rats (LESTER and GREENBERG 1950, MACLEOD 1950), dogs (SMITH 1950, LOOMIS 1950, NEWMAN and PETZOLD 1951, SEIBERT, HUGGINS and BRYAN 1952), mice cats and guinea pigs (SMITH 1950) Acetaldehyde formation and metabolism after pretreatment with TETD were studied by JACOBSEN and co-workers in rabbits When livers from animals which had been pretreated with TETD were perfused with blood to which ethanol had been added there was a marked increase in acetaldehyde in the effluent blood In contrast, a similar perfusion of the hind limbs of an animal pretreated with TETD *showed no ethanol combustion or acetaldehyde formation* (JACOBSEN and LARSEN 1949) This suggested that the increased concentration of acetaldehyde in the blood after TETD + ethanol depended mainly upon an effect of TETD on the function of the liver cells When acetaldehyde was infused into rabbits at a constant rate the concentration of acetaldehyde in the blood reached higher levels in animals pretreated with TETD than in untreated animals (HALD and LARSEN 1949) Results from perfusion studies of isolated organs suggested that this effect of TETD on acetaldehyde metabolism was exerted mainly in the liver (HALD, JACOBSEN and LARSEN 1949 b)

In all of the studies cited above acetaldehyde concentrations in the blood were determined by the methods of STOTZ (1943), or BURBRIDGE, HINE and SCHICK (1950) or modifications of these methods WAGNER (1957), using an enzymic method, also found increased concentrations of acetaldehyde in the blood of rats given TETD + ethanol, but his values were considerably lower than those reported by earlier workers LUNDQUIST (1958), describing another enzymic method for the determination of acetaldehyde, showed that the method of BURBRIDGE *et al* (1950) was subject to interference from acetoacetate, and therefore seriously questioned the specificity of that method Mice given TETD and a small dose of C^{14} labelled ethanol formed only small amounts of radioactive acetaldehyde according to CASIER and POLET (1958)

A large body of evidence thus indicates that TETD exerts an important inhibitory effect on acetaldehyde metabolism but there has not been a correspondingly conclusive demonstration that acetaldehyde accumulation is solely responsible for the clinical manifestations of the TETD-ethanol reaction

CHAPTER I

REVIEW OF EARLIER LITERATURE

In 1948 Danish workers reported that in human subjects pretreated with tetraethylthiuramdisulfide (TETD, disulfiram) small ethyl alcohol (ethanol) doses elicited a characteristic, *transient reaction with a wide variety of manifestations* including conjunctival injection, vasodilation in the face, nausea, hyperventilation and hypotension (HALD, JACOBSEN and LARSEN 1948) A reaction of this type following intake of ethanol had previously been observed in workers exposed to carbon disulfide (HAAS and HEIM 1912) and cyanamide (KOELSCH 1914), and in persons who had eaten the fungus *Coprinus atramentarius* (FISCHER 1945) WILLIAMS (1937) had described a similar reaction to small ethanol doses in workers exposed to tetramethylthiuramdisulfide and tetraethylthiurammonosulfide Noting that this altered response to ethanol forced all exposed workers to become total abstainers, WILLIAMS (1937) had even suggested that these substances should be used to cure alcoholism However, nothing came of this proposal at that time JACOBSEN and co-workers, independently, also realized the great potential importance of the altered response of the organism to ethanol They therefore extended the study of the human pharmacology of TETD (ASMUSSEN *et al* 1948 a, HALD and JACOBSEN 1948), and their work soon led to the introduction of TETD in the treatment of chronic alcoholism (MARTENSEN—LARSEN 1948) under the trade name of Antabuse

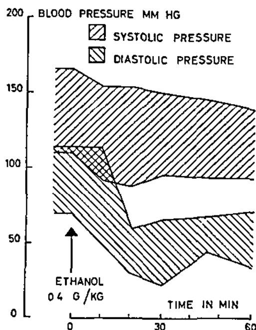
Within a short time after the initial report on the effects of TETD + ethanol *the mode of action* of TETD seemed established No evidence was obtained that TETD in moderate doses influenced the first step in the metabolism of ethanol, its conversion to acetaldehyde (HALD *et al* 1948, HALD, JACOBSEN and LARSEN 1949 a, LOOMIS 1950, NEWMAN and PETZOLD 1951) It was, however, found that in human subjects (HALD and JACOBSEN 1948) and rabbits (LARSEN 1948, KIRCHHEIM 1951, FUJIWARA and KUWANA 1954) given TETD + ethanol the concentration of acetaldehyde in the blood was higher than after ethanol alone Acetaldehyde could also be isolated and chemically identified from the expired air of human subjects (HALD and JACOBSEN 1948) and rabbits (HALD *et al* 1949 a) given TETD + ethanol KJELDGAARD (1949) showed *in vitro* that TETD in concentrations as low as 0 1 µg/ml inhibited aldehyde oxidases from rabbit liver, but had no similar effect on a series of other enzymes including alcohol dehydrogenase The appearance and disappearance of the manifestations of the human TETD-ethanol reaction coin-

cided roughly with the rise and fall in blood acetaldehyde (HINE *et al* 1952), and ASMUSSEN, HALD and LARSEN (1948 b) were able to reproduce several of these manifestations by infusing acetaldehyde into normal human subjects not pretreated with TETD Other findings indicated that acetaldehyde was probably not an intermediate product in normal metabolism (without ethanol) (JACOBSEN 1950) It was concluded from these findings that the TETD-ethanol reaction in man was due to accumulation of acetaldehyde in the blood

An effect of TETD on acetaldehyde metabolism has been shown in a number of further studies on laboratory animals Increases in the concentration of acetaldehyde in the blood after TETD + ethanol were reported in rats (LESTER and GREENBERG 1950, MACLEOD 1950), dogs (SMITH 1950, LOOMIS 1950 NEWMAN and PETZOLD 1951, SEIBERT, HIGGINS and BRYAN 1952) mice, cats and guinea pigs (SMITH 1950) Acetaldehyde formation and metabolism after pretreatment with TETD were studied by JACOBSEN and co workers in rabbits When livers from animals which had been pretreated with TETD were perfused with blood to which ethanol had been added there was a marked increase in acetaldehyde in the effluent blood In contrast, a similar perfusion of the hind limbs of an animal pretreated with TETD showed no ethanol combustion or acetaldehyde formation (JACOBSEN and LARSEN 1949) This suggested that the increased concentration of acetaldehyde in the blood after TETD + ethanol depended mainly upon an effect of TETD on the function of the liver cells When acetaldehyde was infused into rabbits at a constant rate the concentration of acetaldehyde in the blood reached higher levels in animals pretreated with TETD than in untreated animals (HALD and LARSEN 1949) Results from perfusion studies of isolated organs suggested that this effect of TETD on acetaldehyde metabolism was exerted mainly in the liver (HALD, JACOBSEN and LARSEN 1949 b)

In all of the studies cited above acetaldehyde concentrations in the blood were determined by the methods of STOTZ (1943), or BURBRIDGE, HINE and SCHICK (1950) or modifications of these methods WAGNER (1957), using an enzymic method also found increased concentrations of acetaldehyde in the blood of rats given TETD + ethanol, but his values were considerably lower than those reported by earlier workers LUNDQUIST (1958), describing another enzymic method for the determination of acetaldehyde showed that the method of BURBRIDGE *et al* (1950) was subject to interference from acetoacetate, and therefore seriously questioned the specificity of that method Mice given TETD and a small dose of C^{14}-labelled ethanol formed only small amounts of radioactive acetaldehyde according to CASIER and POLET (1958)

A large body of evidence thus indicates that TETD exerts an important inhibitory effect on acetaldehyde metabol...



(HINE et al 1952)

Fig 1 Range of systolic and diastolic blood *pressures before* and up to 60 min after intake of 0 4 g/kg ethanol in 31 alcoholic addicts pretreated with TETD The figure is based on data given by HINE *et al* (1952)

When TETD came into wide clinical use it was confirmed that there was regularly a marked fall in blood pressure during the TETD ethanol reaction (Fig 1), particularly after large amounts of ethanol (RABY and LAURITZEN 1949, HINE *et al* 1952) This hypotension could lead to serious complications, even fatalities (SOLMS 1951, JACOBSEN 1952) It therefore became a major question whether acetaldehyde was responsible for this hypotension

RABY (1955, 1956) made an extensive study of the TETD-ethanol reaction in man, which *confirmed that it was associated with a pronounced fall in* blood pressure He found that the concentration of acetaldehyde in the blood always increased after TETD + ethanol, and was usually higher than after ethanol alone However, after ethanol alone, the concentration of acetaldehyde in the blood occasionally reached the same high levels without producing any of the manifestations characteristic of the TETD-ethanol reaction He concluded from his findings that acetaldehyde was the most probable cause of the symptoms appearing during the TETD-ethanol reaction, but that its concentration in the blood was not solely decisive for the course and degree of the reaction

It is pertinent therefore to consider the pharmacological properties of acetaldehyde *per se* HANDOVSKY (1934, 1936) showed that acetaldehyde, administered intravenously to dogs 1) stimulated the respiration via the chemoreceptors, 2) dilated the bronchial muscles, 3) increased the arterial blood pressure, partly due to a direct effect on peripheral vessels, and 4) increased the pulse rate Adrenal ligation decreased but did not abolish the pressor

effect of acetaldehyde, indicating that this effect was partly but not entirely due to increased adreno-medullary secretion In view of the close metabolic relationship between acetaldehyde and ethanol it may be mentioned in this context that ethanol has been shown to increase the adreno-medullary secretion both in large (KLINGMAN and GOODALL 1957, PERMAN 1961 a) and in small doses (PERMAN 1958 a, 1960, 1961 b ABELIN, HERREN and BERLI 1958)

Further work has confirmed that acetaldehyde has sympathomimetic properties (HERMANN CHATONNET and VIAL 1955 and others) Its pressor effect

[illegible]

TEAGUE and WINGARD 1953), tolazoline and piperoxan (ROMANO, MEYERS and ANDERSON 1954) TEAGUE and WINGARD (1953) and ROMANO *et al* (1954) were apparently the first to suggest that acetaldehyde acts via adrenergic transmission mechanisms PERMAN (1958 b) observed that acetaldehyde transiently increased the catecholamine level in the adrenal vein blood of cats after splanchnic nerve section suggesting that acetaldehyde can exert a direct releasing effect on catecholamine-containing cells EADE (1959) studied the effects of acetaldehyde on the blood pressure and on the nictitating membrane of spinal cats with ligated adrenals and found that its sympathomimetic effects disappeared after treatment with reserpine which depletes the catecholamine stores (CARLSSON *et al* 1957) These results suggest that acetaldehyde, like tyramine (BURN and RAND 1958), exerts its sympathomimetic action partly by releasing catecholamines from tissue stores other than the adrenal medulla

Other pharmacological effects of acetaldehyde have also been demonstrated in animal experiments TROQUET and LECOMTE (1960) reported that acetaldehyde but neither TETD nor ethanol alone could liberate histamine from various rat tissues *in vitro* Other *in vitro* studies showed that acetaldehyde, but not ethanol exerted a moderate uncoupling effect on oxidative phosphorylations (TRUITT BELL and KRANTZ 1956), and PERMAN (1962 a) observed that acetaldehyde in small doses increased the oxygen uptake of anesthetized rabbits This suggested that acetaldehyde, formed from ethanol might be the cause of the moderate increase (10—15 %) in oxygen uptake which is seen after small ethanol doses (0 1—0 2 g/kg) under similar conditions (PERMAN 1961 c 1962 b c) The relation if any, of these various effects of acetaldehyde to the TETD-ethanol reaction is not known

In view of the sympathomimetic properties of acetaldehyde it has been difficult to attribute the hypotension during the human TETD ethanol reaction to the increased acetaldehyde concentration in the blood Acetaldehyde was even at one time given clinically (*per os*) with some success as a circulatory stimulant in shock (GYORGY 1932) As mentioned ASMUSSEN *et al* (1948 b) were able to produce several of the symptoms which are characteristic of the TETD ethanol reaction (hyperventilation tachycardia, vasodilation in the

face) by infusing acetaldehyde into normal human subjects, but they gave no data on the blood pressure RABY (1955, p 45) tried to reproduce the change in systemic pressure by infusing larger doses of acetaldehyde, but his subjects experienced very severe pain during the infusion so this attempt had to be given up Further studies on the effects of acetaldehyde in man have apparently not been carried out

Some earlier results have indicated that the effect of acetaldehyde on the blood pressure of animals is altered by pretreatment with TETD CHRISTENSEN (1951) confirmed the earlier observation that injection of acetaldehyde in dogs gave a biphasic pressure response resembling closely that produced by adrenaline He also found that pretreatment with TETD exaggerated the hypotensive phase of the response to both adrenaline and acetaldehyde, and therefore assumed that ' the action of TETD is to alter the response of specific sympathomimetic receptor cells to the *sympathomimetic mechanism of acetaldehyde* or l-epinephrine" However, FEINGOLD (1954) found that while the pressor action of injected acetaldehyde in dogs was diminished after pretreatment with TETD, the prolongation of the depressor phase was much less than that reported by CHRISTENSEN (1951) FEINGOLD (1954) also noted that pretreatment with TETD did not alter the blood pressure response of dogs to injected adrenaline, noradrenaline, histamine or acetylcholine

Several attempts by earlier investigators to reproduce the chief manifestations of the human TETD ethanol reaction with ethanol in animals pretreated with TETD have been unsuccessful LARSEN (1948) administered ethanol to rabbits pretreated with TETD, and saw an increase in ventilation in some instances but no changes in blood pressure or heart rate SMITH (1950) reported that the reactions of dogs given TETD + ethanol were similar to those observed in humans", but the details of this work have apparently not been published, and CHILD (1951) and SEIBERT *et al* (1952) were unable to demonstrate any TETD-ethanol reaction in dogs ZIEGLER and MEYER (1959) reported that an equivalent to the human TETD-ethanol reaction could not be produced in the cat except under extreme conditions (after liver poisoning with CCl_4) No equivalent to the human TETD-ethanol reaction thus seemed to be produced in animals Certain aspects of the pharmacodynamic effects of TETD + ethanol have, however, been studied in animals Several workers observed that the toxicity of ethanol (LARSEN 1948, LECOQ 1949, CHILD, CRUMP and LEONARD 1952) and of acetaldehyde (DE JONGH 1952) were augmented by pretreatment with TETD CZYZYK (1952) found that the respiratory gas exchange of rabbits was increased by ethanol alone but decreased after TETD + ethanol, and STAUB (1955) obtained similar results with mice FERGUSON (1956) found that the bleeding weight (the weight of the blood in the large systemic arteries and veins in relation to body weight) was reduced in rats and mice given TETD + ethanol, and used this criterion to screen other drugs for TETD-like activity

This review shows that the pharmacodynamic mechanism of the TETD-ethanol reaction is still obscure to a large extent Although it seems certain that increased amounts of acetaldehyde are present in the blood during the reaction its exact role is still not clear An important obstacle in the previous studies of the TETD ethanol reaction has been that an altered response to small ethanol doses apparently never has been convincingly demonstrated in animals pretreated with TETD in spite of several attempts This particularly holds for the marked fall in blood pressure which is such a prominent and important manifestation of the human TETD-ethanol reaction Extensive studies of the reaction have been carried out in man, but no detailed experimental analysis has, for obvious reasons been possible in those studies Furthermore, the fact that human subjects in shock do not lend themselves to more than a minimum amount of experimental manipulation has made it difficult to evaluate the many counter measures which have been proposed against the serious circulatory complications which may occur during TETD ethanol reactions

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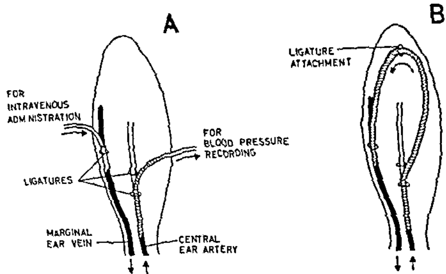


Fig 2 Schematic drawing of preparation for repeated recordings of arterial blood pressure in the rabbit without general anesthesia
A Ear (dorsal surface) during experiment
B Ear between experiments

and expiratory valves The femoral artery was cannulated for continuous recording of the systemic arterial pressure, and the ipsilateral femoral vein was used for infusions and injections Operative wounds were treated with small amounts of a local anesthetic (XYLOCAINE, Astra, 2 %) The animal was given heparin (Vitrum) in amounts adequate for preventing coagulation (4,000 units i v) The whole preparation could, as a rule, be completed within 20 min and with loss of less than 1 cc of blood The systemic arterial pressure was recorded by a mercury manometer, writing on a smoked drum The ventilation [illegible]
air was colle [illegible]
Thus spirom- [illegible] emptied via a magnetic valve at regular intervals of 15 sec or 5 sec In this way the expired volume per time unit was recorded The body (colonic) temperature was checked during experiments, and was maintained between 38° C and 39° C

Un-anesthetized rabbits

In order to record the blood pressure intermittently for several days *in* un-anesthetized rabbits, the following technique was devised

The animal was placed in a restraining box where it could sit or lie comfortably during the experiment *Under local* infiltration anesthesia (XYLOCAINE, 2 %) thin polyethylene catheters (INTRAMEDIC PE 50 outer

CHAPTER II

GENERAL METHODS

Male, albino rabbits weighing 2—4 kg were used. The animals received a standard diet (barley, hay, turnip) and water *ad libitum* before and between experiments.

Tetraethylthiuramdisulfide (TETD)[1] was administered by stomach tube in an aqueous gum arabic suspension. The suspending medium was a sucrose solution (63 % w/w) containing 24 % (w/v) gum arabic. In a majority of rabbits a dose of 1 g TETD (10 ml of a suspension containing 0.1 g/ml) was administered twice with an interval of 24 hours, and the experiments were started 1—5 hours after the last TETD dose. For convenience, animals so treated are called "pretreated rabbits", and are contrasted to "un-pretreated rabbits" throughout this paper. Some rabbits were pretreated with smaller doses of TETD or given TETD free gum arabic solution in corresponding volumes and according to the same schedule.

Ethanol[2] was diluted with saline (0.9 %) to required concentrations. In the majority of experiments an intravenous ethanol dose of 4.4 mmoles/kg was used (2.5 ml/kg of a solution containing 1.74 mmoles/ml). This dose equals 0.2 g/kg (1 mmole ethanol = 0.046 g). Acetaldehyde[3] solutions were made up with saline and were stored at + 4° C. Acetaldehyde doses are also given in terms of mmoles/kg (1 mmole acetaldehyde = 0.044 g). Pure saline was used for control purposes. When, in the same experiment, several ethanol or acetaldehyde doses were given the administered volumes were kept constant and concentrations appropriately varied. All infusions were made at constant rates of 1.0 ml/min or 0.18 ml/min with a motor-driven device. Unless specified, the former infusion rate was used. All solutions were warmed to room temperature in the syringe before administration.

Anesthetized rabbits

In anesthetized rabbits the experimental procedure was as follows. The animal was anesthetized with urethane (1.4 g/kg i.v.) and placed on its back on an operating table. The trachea was cannulated and connected to inspiratory

[1] Supplied by Dumex Ltd. or Synthetic Ltd., Denmark (purity > 99.5 %). No differences were seen between the effects of the two substances.
[2] Supplied by AB Vin- och Spritcentralen, Sweden (purity 99.5 % v/v).
[3] Supplied by Merck Ltd., Germany (purity > 99 %) or KEBO Ltd., Sweden (purity > 98 %). No differences were seen between the effects of the two substances.

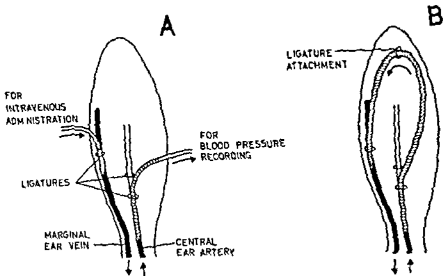


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[illegible] emptied via a magnetic valve at regular intervals of 15 sec or 5 sec In this way the expired volume per time unit was recorded. The body (colonic) temperature was checked during experiments, and was maintained between 38° C and 39° C

Un anesthetized rabbits

In order to record the blood pressure intermittently for several days in un anesthetized rabbits, the following technique was devised

The animal was placed in a restraining box where it could sit or lie comfortably during the experiment Under local infiltration anesthesia (XYLOCAINE, 2 %) thin polyethylene catheters (INTRAMEDIC PE 50 outer

diameter 0 97 mm, inner diameter 0 58 mm) were inserted into the central artery and marginal vein of one ear and fixated with ligatures (Fig 2 A) The procedure could, with some experience, be carried out within 15 min and apparently caused the animal little discomfort. Aseptic conditions were not observed The arterial catheter was filled with heparin solution (2,000 units/ml), and a single dose of heparin (4,000 units i v) was given at the beginning of the experiments The ear artery pressure was recorded either by a mercury manometer writing on a smoked drum, or via a pressure transducer (ELEMA) coupled to an ink-writer (VARIAN MODEL G 10 RECORDER)

At the end of experiments the catheters in the artery and vein were connected (Fig 2 B), so that the blood circulated (at a slow flow rate) in an extracorporeal loop The loop was attached to the ear by a single ligature In the present work the interval between the blood pressure recordings was usually 24 hours After 24 hours there was no longer any blood flow in the loop, but the catheters were easily "washed" with saline In no instance was the loop "disconnected" or otherwise damaged by the animal between experiments The rabbits always rested very quietly during the experiments, indicating that they did not feel any pain

No infection or signs of necrosis were observed in the ears of rabbits with loops maintained up to 7 days after the initial preparation This must be due to the well developed collateral circulation in the rabbit ear

Outline of the present investigation

The present investigation was carried out according to the following general plan First a study was made (Chapter III) of the effects of ethanol on the blood pressure and ventilation of un pretreated and pretreated rabbits to establish whether in fact an equivalent to the human TETD ethanol reaction could be demonstrated in the rabbit Then a study was undertaken to determine the corresponding effects of acetaldehyde (Chapter IV) Finally, experiments were designed to obtain some information about the mechanism of the ethanol-induced fall in blood pressure which occurred in pretreated rabbits (Chapter V)

All individual results presented in this paper were obtained in experiments on at least 3 different animals unless otherwise stated

CHAPTER III

EFFECT OF ETHANOL IN RABBITS PRETREATED WITH TETRAETHYLTHIURAMDISULFIDE

Pretreatment with TETD

The general condition of the rabbits was not appreciably affected by pretreatment with TETD, as judged from their appearance and behavior Their initial blood pressure and ventilation levels were in the same range as those of un pretreated animals (Table I) No signs of altered sensitivity to the standard anesthetic urethane dose were seen

Anesthetized animals

When *un pretreated* rabbits were given the standard ethanol dose (4 4 mmoles/kg infused i v during 5—10 min) a slight, gradual decrease in blood pressure and a concomitant, moderate ventilatory increase were observed during 2 hours after the end of the ethanol infusion (Fig 3) However, these changes were also seen when a corresponding volume of saline was infused into unpretreated animals (2 exp) (Fig 3)

In contrast *pretreated* rabbits regularly responded within 30 min of the standard ethanol dose with, 1) a fall in blood pressure from initial levels of 90—110 mm Hg down to about 60 mm Hg, and 2) a clearcut ventilatory increase (Fig 3) No similar effects were produced by infusion of saline only The fall in blood pressure and the increase in ventilation following ethanol were significantly greater in pretreated than in un pretreated rabbits (Table I) Fig 4 shows the effects of ethanol on blood pressure and ventilation in a pretreated rabbit In no case did ethanol (4 4 mmoles/kg) fail to produce these circulatory and respiratory effects in pretreated rabbits (> 20 exp) The ethanol induced hypotension was usually long lasting, the "pre-ethanol" pressure level being reattained after 3—4 hours A few animals, however, did not recover, but died in a state of severe hypotension

Ethanol doses of 1 1—4 4 mmoles/kg were, as a rule, needed to produce in pretreated animals the circulatory and respiratory effects described above With a larger ethanol dose (18 mmoles/kg) the hypotension became even more pronounced However, in a few animals smaller ethanol doses could produce the same response pattern Fig 5 shows an animal which reacted to only 0 28 mmole/kg ethanol (a corresponding infusion of saline being without effect)

The effects of pretreatment with smaller doses of TETD were also studied Pretreatment with 0 1 g TETD for 2 days (2 exp) or with 0 01 g TETD for

Table I Effect of intravenous infusion of 4 4 mmoles/kg (0 2 g/kg) ethanol on blood pressure and ventilation in un pretreated and pretreated, urethane anesthetized rabbits The mean decrease in blood pressure and the mean increase in ventilation are significantly larger (blood pressure fall P< 0 001 ventilatory increase P< 0 01) in the pretreated group (6—10) than in the un pretreated group (1—5)

Rabbit No	Weight kg	Pretreatment with TETD g	Initial levels		Change 30 min after end of ethanol infusion 4 4 mmoles/kg	
			Blood pressure mm Hg	Ventilation ml/min	Blood pressure mm Hg	Ventilation ml/min
1	2 5	0	110	550	— 15	+ 150
2	2 7	0	85	650	— 5	+ 150
3	2 8	0	105	700	— 15	+ 150
4	3 1	0	105	650	— 5	+ 100
5	3 2	0	115	800	— 10	+ 50
Mean			**104**	**670**	**— 10**	**+ 120**
6	2 6	1 + 1	105	500	— 45	+ 300
7	2 8	1 + 1	90	900	— 30	+ 300
8	2 9	1 + 1	110	800	— 40	+ 450
9	3 1	1 + 1	110	500	— 45	+ 650
10	3 1	1 + 1	95	900	— 30	+ 450
Mean			**102**	**720**	**— 38**	**+ 430**

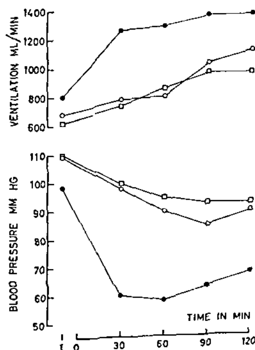


Fig 3 Mean systemic blood pressure and ventilation levels in 3 groups of anesthetized rabbits before (I) and up to 120 min after intravenous administration of saline or ethanol
□ Un pretreated rabbits — saline infusion (2 exp)
○ Un pretreated rabbits — ethanol infusion (4 4 mmoles/kg) (4 exp)
● Pretreated rabbits — ethanol infusion (4 4 mmoles/kg) (4 exp)

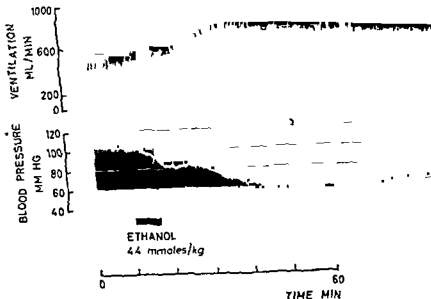


Fig 4 Pretreated rabbit Urethane anesthesia Ethanol, 4 4 mmoles/kg infused i v during signal.

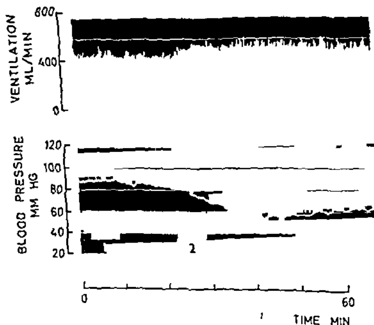


Fig 5 Pretreated rabbit. Urethane anesthesia
1 Saline infusion (6 cc i v) 2 Ethanol infusion, 0 28 mmole/kg (13 mg/kg) i v

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			Blood pressure mm Hg	Ventilation ml/min	Blood pressure mm Hg	Ventilation ml/min
1	2 5	0	110	550	— 15	+ 150
2	2 7	0	85	650	— 5	+ 150
3	2 8	0	105	700	— 15	+ 150
4	3 1	0	105	650	— 5	+ 100
5	3 2	0	115	800	— 10	+ 50
Mean			**104**	**670**	**— 10**	**+ 120**
6	2 6	1 + 1	105	500	— 45	+ 300
7	2 8	1 + 1	90	900	— 30	+ 300
8	2 9	1 + 1	110	800	— 40	+ 450
9	3 1	1 + 1	110	500	— 45	+ 650
10	3 1	1 + 1	95	900	— 30	+ 450
Mean			**102**	**720**	**— 38**	**+ 430**

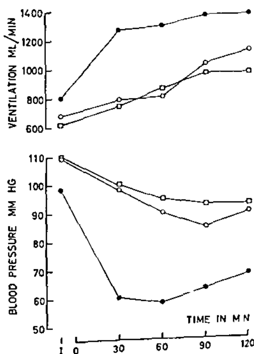


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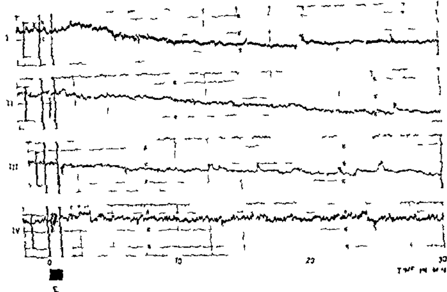


Fig 8 Pretreated rabbit No general anesthesia Recordings of ear artery pressure on 4 consecutive days Scale upper limit 100 mm Hg, lower limit 60 mm Hg
At E (between markings) ethanol infusion (infusion time 1 min), 4 4 mmoles/kg i v
I First day, 4 hours after the second TETD dose
II Second day
III Third day
IV Fourth day

(Fig 7) similar to that seen in anesthetized animals Failure of *ethanol to* produce hypotension has *not been* observed in pretreated, un-anesthetized rabbits However, no un-anesthetized, pretreated rabbit died *after the standard ethanol dose*

The altered blood pressure response to ethanol persisted for about 3 d[illegible]

[illegible] ethanol produced hypotension A similar hypotension was seen when ethanol was *administered 24* hours after a single TETD dose of 0 5 g (2 exp)

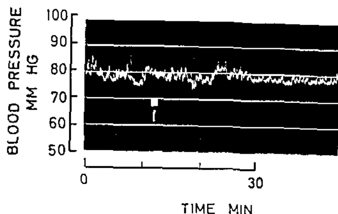


Fig 6 Un pretreated rabbit No general anesthesia Recording of ear artery pressure I Ethanol infusion (infusion time 2 min), 4 4 mmoles/kg i v



Fig 7 Pretreated rabbit No general anesthesia Recording of ear artery pressure
I Ethanol infusion (infusion time 2 min) 4 4 mmoles/kg i v
Interval of 60 min between record A and B

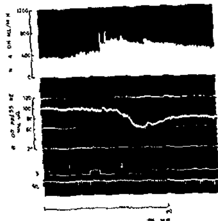
2 days (2 exp) did not alter the response to the standard ethanol dose, and pretreatment with TETD free gum arabic solution (2 exp) was likewise without effect

Un-anesthetized animals

In order to determine the influence of general anesthesia and operative procedure on the effects of TETD + ethanol, experiments were devised where the effect of ethanol on the blood pressure could be studied without general anesthesia and with only minor preparations It was found in control experiments that the blood pressure levels of both pretreated and un-pretreated animals were stable at levels of 85—100 mm Hg over long periods (4 hours) except for occasional short-lasting fluctuations

In *un-pretreated* animals the standard ethanol dose (4 4 mmoles/kg) did not affect the blood pressure (Fig 6) In *pretreated* rabbits, however, the same ethanol dose regularly produced a marked, transient fall in blood pressure

Fig 10 Pretreated rabbit Urethane anesthesia
1 Saline infusion (3 6 ml) i v during 20 min
2 Acetaldehyde infusion
0 030 mmole/kg/min i v during 20 min



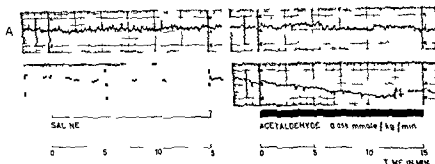


Fig 11 A Unpretreated rabbit B Pretreated rabbit
No general anesthesia Recordings of ear artery pressure Scale upper 1 unit 100 mm Hg lower 1 unit 60 mm Hg
Between vertical lines 15 min i v infusions of saline (2 7 ml) and of acetaldehyde 0 058 mmole/kg/min

duration There was also a marked ventilatory increase The secondary fall in blood pressure was usually more pronounced in pretreated animals, but this was not a consistent finding

Unanesthetized animals

The effect of acetaldehyde infusion on the blood p...

CHAPTER IV

EFFECT OF ACETALDEHYDE IN RABBITS PRETREATED WITH TETRAETHYLTHIURAMDISULFIDE

Anesthetized animals

In one group of experiments acetaldehyde was infused (0 18 ml/min) during 15—20 min at rates of 0 03—0 06 mmole/kg/min In *un-pretreated* animals there was a ventilatory increase, but the systemic pressure level was mainly unaffected (Fig 9) In *pretreated* animals a similar ventilatory increase was seen, but there was also a fall in blood pressure from initial levels of 90—110 mm Hg down to 60—70 mm Hg during the infusion (Fig 10) When the infusion was discontinued the blood pressure as a rule started to increase within 10 min

In another group of experiments small amounts of acetaldehyde (0 22—0 87 mmole) were administered by rapid intravenous injection Both in un pretreated and pretreated animals this produced a brief initial pressor response (10—20 mm Hg) followed, as a rule, by a secondary fall in blood pressure of longer

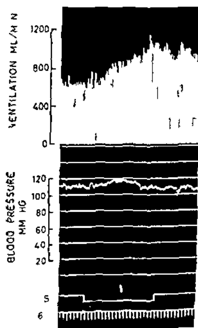


Fig 9 Un pretreated rabbit Urethane anesthesia I Acetaldehyde infusion 0 028 mmole/kg/min i v during 20 min

CHAPTER V

MECHANISM OF THE HYPOTENSIVE ACTION OF ETHANOL IN RABBITS PRETREATED WITH TETRAETHYLTHIURAMDISULFIDE[1]

1 Special methods

All animals used in the experiments described in this chapter were anesthetized with urethane (1 4 g/kg i v) The following special methods were employed in some of these experiments

Neuromuscular blockade was produced in some animals with an intravenous injection of 2—4 mg decamethonium iodide (Burroughs Wellcome) or decamethonium bromide (SYNCURINE, Burroughs Wellcome), and the animals were subsequently ventilated with a STARLING pump The rate and volume of the arterial ventilation were adjusted so as to equal the preceding spontaneous ventilation

In some experiments it was studied how pretreated rabbits reacted to bilateral carotid occlusion (30 sec) and to administration of noradrenaline before and during the ethanol induced period of hypotension Noradrenaline was either rapidly injected or infused slowly intravenously Doses are expressed in terms of the hydrochloride of the amine In other experiments the animals were subjected for short periods (5—10 min) to the following gas mixtures 6 5 % CO_2 in O_2 pure oxygen and 10 % O_2 in N_2

The circulatory and respiratory effects of the standard ethanol dose in pretreated rabbits were also studied in experiments where the following drugs had been given 30—90 min prior to ethanol

atropine sulphate (Atropini sulfas Sw Ph XI)
mepyramine maleate (ANTHISAN, May & Baker)
promethazine hydrochloride (LERGIGAN, Recip)
chlorpromazine hydrochloride (HIBERNAL, Leo)
hexamethonium bromide (VEGOLYSEN, May & Baker)

The following parameters were determined in other studies (though not all in the same experiment)

1) systemic arterial pressure
2) heart rate
3) respiratory rate
4) pH of the arterial blood and

[1] Some of the experiments described in this chapter were performed in collaboration with Dr S BYGDEMAN and Dr N SJÖSTRAND and will be included in a separate report (BYGDEMAN PERMAN and SJÖSTRAND to be published)

in a pretreated animal (Fig 11) An increase in rate and depth of ventilation could be observed both in the pretreated and the un-pretreated animal when acetaldehyde was administered No other gross changes in appearance or behavior of the animals were noted during these experiments

CHAPTER V

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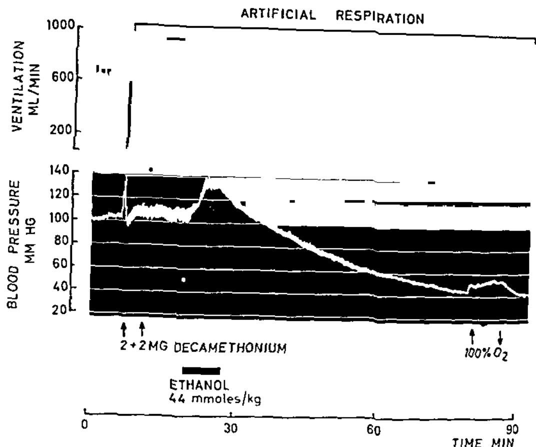


Fig 12 Pretreated rabbit Urethane anesthesia Neuromuscular blockade produced with 4 mg decamethonium i v at beginning of experiment Artificial ventilation Ethanol 4 4 mmoles/kg infused i v during signal Animal ventilated with oxygen for 6 min at end of experiment

5) arterial pressure in the hind limb, perfused at a constant flow rate with blood from the same animal (local vascular resistance)

The operative procedure was as follows After insertion of a tracheal cannula the left femoral artery was dissected free and ligated about 1 cm below the *inguinal ligament* The artery was then cannulated on both sides of the ligature and the cannulas connected with a polyethylene tube The blood flow in this exteriorized arterial loop was kept constant by a pump (SIGMAMOTOR) Immediately distal to the pump was determined the local arterial pressure (local vascular resistance) of the perfused region and/or the arterial pH The flow rate in the loop was adjusted at the start of the experiment so that the local (perfusing) pressure equalled the systemic pressure level The capacity of the loop (in ml) was determined It is of *importance for the interpretation of* some of the present results to emphasize that with this technique a substance *appearing in the systemic circulation* will reach the perfused vascular region later than it reaches other peripheral regions owing to the delay in the loop The systemic arterial pressure was recorded from the contralateral femoral

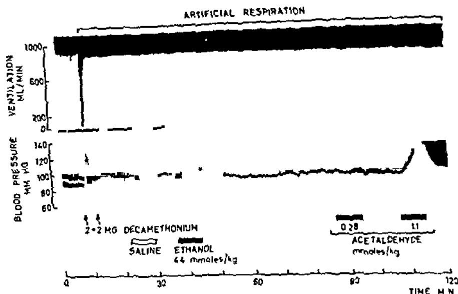


Fig 13 Un pretreated rabbit Urethane anesthesia Neuromuscular blockade produced with 4 mg decamethonium i v at beginning of experiment Artificial ventilation First ethanol (4 4 mmoles kg) and a corresponding volume (7 cc) of saline were infused i v Later two different doses of acetaldehyde were infused

artery, and the femoral vein on that side was used for infusions and injections The systemic and local arterial pressures and the tracheal pressure variations were recorded on a four channel polygraph (GRASS) via suitable pressure transducers (STATHAM) The heart rate was recorded on the polygraph through an ordinate writer (GOLDSCHMIDT and LINDGREN 1962) Arterial pH was recorded on the polygraph or followed on a pH meter (RADIOMETER PHM 22) The part of the loop which contained the pH-electrode was kept at a constant temperature (39 °0 C) by a water bath, and the rest of the loop was warmed by an air current In two experiments the sympathetic chain was sectioned at the level of L_1—L_3, and in one of these experiments the peripheral part was stimulated intermittently during 20 sec periods with a stimulus strength of 7 V a stimulus duration of 1 msec and a frequency of 2/sec

In vitro experiments

Intestines from pretreated and un pretreated rabbits were used The organs were removed during urethane (1 4 g/kg) anesthesia

Pieces 3—4 cm long of the jejunum were placed in a 50 ml organ bath containing TYRODE solution at 37° C and aerated with 6 5 % CO_2 in O_2

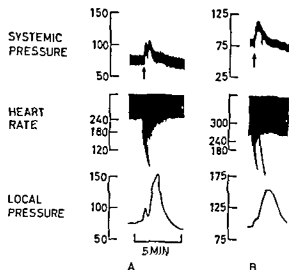


Fig 14 Effect of rapid intravenous injection (at arrows) of 0 87 mmole acetaldehyde in an un pretreated (A) and a pretreated (B) rabbit (body weights 3 kg) Both animals are ventilated artificially at a constant rate after neuromuscular blockade Urethane anesthesia From above downwards systemic arterial pressure in mm Hg, heart rate in beats per min, local arterial pressure in left hind limb, perfused at constant flow rate with blood from the same animal time scale

Contractions were recorded on a smoked drum with a standard frontal writing lever Most experiments were performed on FINKLEMAN preparations (FINKLEMAN 1930) The periarterial (sympathetic) nerve was placed on stimulating electrodes and covered with liquid paraffin It was stimulated for 10—20 sec every 2 or 3 min with supramaximal strength (15 V), the frequency being in the range of 10—20 per sec and the duration 2 msec The stimulation was programmed" by a laboratory timer (PERMAN and PERSSON, to be published) Ethanol and acetaldehyde (both diluted in TYRODE solution) were added directly to the bath to required concentrations

2. Results

Artificial ventilation

As it has frequently been observed in pretreated rabbits given ethanol or acetaldehyde that the peak ventilatory increase preceded the maximal fall in blood pressure, experiments were carried out to establish whether the fall in blood pressure was secondary to increased ventilation

Ethanol (4 4 mmoles/kg) was administered to pretreated and un-pretreated, anesthetized rabbits, artificially ventilated at a constant rate after neuromuscular blockade There was the usual, *marked fall in blood pressure in* pretreated animals also under these conditions (2 exp) (Fig 12), whereas no change was seen in un-pretreated animals (2 exp) (Fig 13) In one of the pretreated animals a marked, transient rise in systemic pressure was noted during the ethanol infusion (Fig 12)

Infusion of *acetaldehyde* (0 03—0 06 mmole/kg/min) also caused a fall in blood pressure in pretreated rabbits when artificially ventilated at a constant rate after neuromuscular blockade (2 exp) In un-pretreated animals there

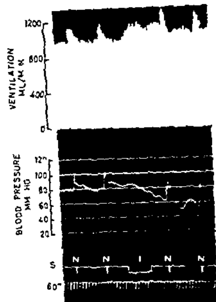


Fig 15 Pretreated rabbit. Urethane anesthesia
N Noradrenaline 20 μg i v
I Ethanol infusion 4 4 mmoles/kg i v

was no corresponding hypotensive effect (2 exp) Rapid injection of acetaldehyde (0 22—0 87 mmole, i v) produced mainly a brief pressor response both in un pretreated and pretreated, artificially ventilated animals (Fig 14, see also Fig 13)

Carotid occlusion reflex and noradrenaline

These experiments were undertaken to study the ability of the systemic pressure to increase in response to stimulation of the baroreceptor system or to administration of sympathetic transmitter during the period of hypotension induced by ethanol A rise (~ 10 mm Hg) in systemic pressure was still observed in response to bilateral carotid occlusion The ability of noradrenaline to produce a rise in systemic pressure was not impaired during the ethanol-induced hypotension whether injected (Fig 15) or infused at a slow rate. The pre ethanol pressure level could accordingly be restored by noradrenaline infusions

Hypercapnia oxygen and hypoxia

These experiments were performed to study the effect of hypercapnia and hypoxia both powerful stimuli of the vasoconstrictor system at the central level on the systemic pressure during the hypotension During the ethanol-induced hypotension the systemic pressure increased temporarily to its "pre-ethanol level when the animal was subjected to hypercapnia (6 5 % CO_2

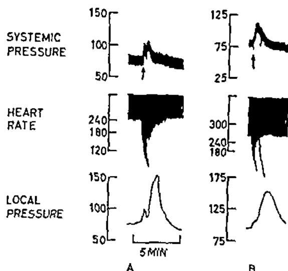


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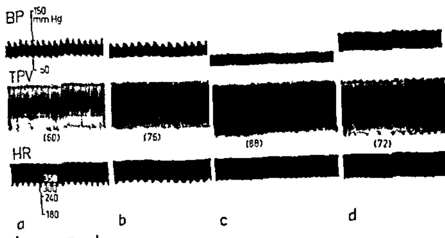


Fig 17 Pretreated rabbit. Urethane anesthesia From above downwards (BP) systemic arterial pressure in mm Hg (TPV) tracheal pressure variations figure within brackets denotes respiratory rate per min (HR) heart rate in beats per min time scale 1 min

a. Control period

b. Immediately after ethanol infusion 4 4 mmoles/kg i v

c 25 min after end of ethanol infusion

d 60 min » » » » »

(5 mg/kg i v) (2 exp with each drug) Rabbits treated with hexamethonium (2—5 mg/kg i v) had pre ethanol systemic pressure levels of 70—80 mm Hg In these animals ethanol induced a marked fall in blood pressure (down to 40—50 mm Hg) Rabbits given chlorpromazine (1 or 5 mg/kg i m) (2 exp) became somnolent They required less than the standard urethane dose for surgical narcosis and their pre ethanol systemic blood pressure levels were low (70—80 mm Hg) However infusion of the standard ethanol dose elicited a fall in blood pressure (> 20 mm Hg) as well as a ventilatory increase

Respiratory rate, heart rate and arterial pH

These experiments were performed to obtain information about respiratory rate heart rate and rhythm and arterial pH following administration of the standard ethanol dose to pretreated rabbits There was a marked increase in respiratory rate concomitant with the ethanol induced fall in blood pressure (1 exp) (Fig 17) No arrhythmia or marked change in heart rate was observed A slight increase in heart rate was however noted during the first 20 min after administration of ethanol (Fig 18—20) During the same period there was a slight increase in arterial pH but afterwards the pH decreased gradually below its pre ethanol level (Fig 18)

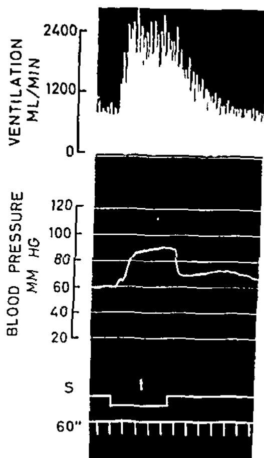


Fig 16 Pretreated rabbit Urethane anesthesia Initial blood pressure level low due to a previous (30 min) ethanol infusion (4 4 mmoles/kg i v)
I Breathing 6 5 % CO_2 in O_2

in O_2) (Fig 16). Pure oxygen produced no similar effect. Hypoxia (10 % O_2 in N_2) could also produce a marked, temporary rise in systemic pressure during the period of hypotension

Hexamethonium, atropine, mepyramine, promethazine and chlorpromazine

In these experiments it was studied if the response to ethanol in pretreated rabbits could be blocked by drugs Hexamethonium is commonly used to produce ganglionic blockade Atropine is known to block cholinergic vasodilation Mepyramine and promethazine are antihistaminics, the latter also has a central depressant action Chlorpromazine, finally, is mainly a central depressant drug, and has been recommended clinically to counteract the malaise which accompanies severe TETD-ethanol reactions

The effects of the standard ethanol dose (4 4 mmoles/kg) on the systemic pressure and ventilation of pretreated rabbits described above (Chapter III) were not appreciably affected by the previous (30—90 min) administration of atropine (3 mg/kg i v), mepyramine (3 mg/kg i v), or promethazine

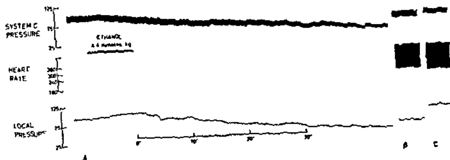


Fig 19 Pretreated rabbit Urethane anesthesia From above downwards systemic arterial pressure in mm Hg, signal, heart rate in beats per min, local arterial pressure in mm Hg in left hind limb, perfused at constant flow rate (6 ml min) with blood from the same animal, time scale in min Capacity of loop 6 ml

B 80 min after end of ethanol infusion

C 130 min after end of ethanol infusion.

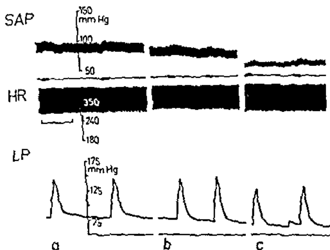


Fig 20 Pretreated rabbit Urethane anesthesia *From* above downwards (SAP) systemic arterial pressure in mm Hg, signal, (HR) heart rate *in beats per min*, time scale 1 min, (LP) local arterial pressure in mm Hg in the left hind limb, perfused at a constant *flow rate* (4 ml/min) with blood from *the same* animal Sympathetic nerves to the perfused *region cut*.

a Control period

b Immediately after ethanol *infusion*, 4 4 mmoles/kg i v

c 40 min after end of ethanol infusion

At signals electrical stimulation of *the left* sympathetic chain between L4 and L5

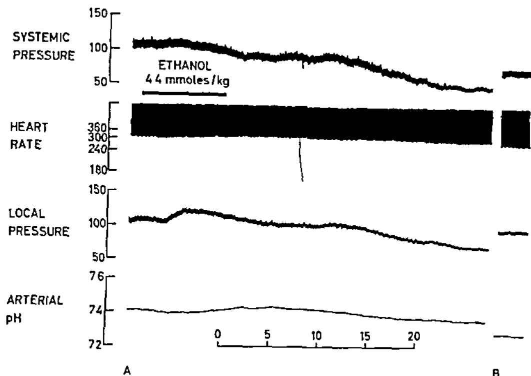


Fig 18 Pretreated rabbit Urethane anesthesia From above downwards systemic arterial pressure in mm Hg, signal, heart rate in beats per min, local arterial pressure in mm Hg in the left hind limb, perfused at constant flow rate (6 ml/min) with blood from the same animal, arterial pH, time scale in min Capacity of loop 9 ml
B 80 min after end of ethanol infusion

Vascular resistance in the hind limb and stimulation of vasoconstrictor nerves

These experiments were performed to obtain information about the peripheral vascular resistance during the hypotension It was also of interest to see if the response to postganglionic stimulation of sympathetic nerves was altered *during the hypotension*

The vascular resistance in the perfused hind limb of pretreated rabbits decreased during the ethanol-induced fall in blood pressure (Fig 18—19) A decrease in vascular resistance following administration of ethanol was also seen in experiments where the sympathetic nerves to the perfused region were cut (Fig 20) (2 exp) It was noted that the fall in the systemic pressure preceded the fall in local pressure of the perfused hind limb, and that this time difference was most pronounced when an extracorporeal loop with a large capacity was used *This strongly suggested that the decrease in vascular resistance* was primarily due to a blood-borne factor with peripheral action No impairment of the ability of the vascular bed to constrict in response to electrical stimulation of the sympathetic chain was observed during the hypotensive period (1 exp) (Fig 20)

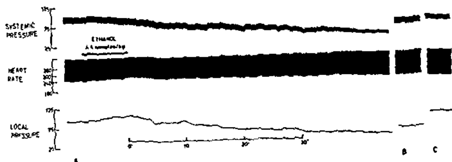


Fig 19 Pretreated rabbit Urethane anesthesia From above downwards systemic arterial pressure in mm Hg, signal, heart rate in beats per min, local arterial pressure in mm Hg in left hind limb, perfused at constant flow rate (6 ml/min) with blood from the same animal; time scale in min Capacity of loop 6 ml.
B 80 min after end of ethanol infusion
C. 130 min after end of ethanol infusion

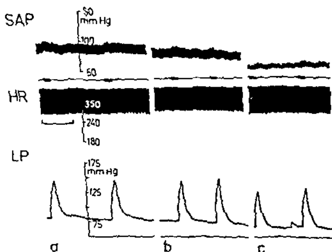


Fig 20 Pretreated rabbit Urethane anesthesia From above downwards (SAP) systemic arterial pressure in mm Hg, signal, (HR) *heart rate* in beats per min, time scale 1 min, (LP) local arterial pressure *in* mm Hg *in the left hind* limb, perfused at a constant flow rate (4 ml/min) with blood from the *same animal* Sympathetic nerves to the perfused region cut.
a Control period
b Immediately after ethanol infusion, 4 4 mmoles/kg i v.
c 40 min after end of ethanol infusion
At signals electrical stimulation of the left sympathetic chain between L4 and L5

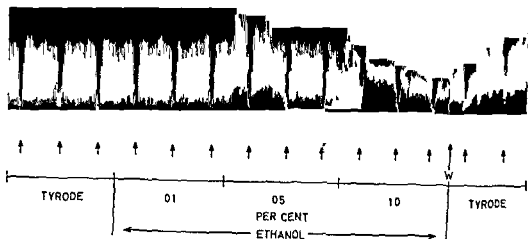
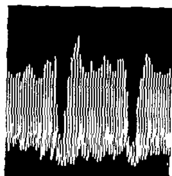


Fig 21 Contractions of isolated jejunum strip from pretreated rabbit without and in the presence of ethanol in 3 different concentrations (0 1, 0 5 and 1 0 per cent) At arrows the periarterial nerves were electrically stimulated Bath volume 50 ml
W washing of bath





A B

Fig 22 *Contractions of isolated jejunum strip from pretreated rabbit without* (A) and in the presence of acetaldehyde *(0 01* per cent w/v) (B) At arrows the periarterial nerves were *electrically stimulated* Bath volume 50 ml

Experiments were also performed where the effect of rapid intravenous injection of small amounts of *acetaldehyde,* (0 44—0 87 mmole i v) on the local vascular resistance in the hind limb was studied These animals were artificially ventilated at a constant rate after neuromuscular blockade Injections of acetaldehyde elicited a marked, transient increase in vascular resistance, roughly coinciding with the increase in systemic pressure, both in un pretreated and in pretreated animals (Fig 14)

In vitro results

In vitro experiments were conducted on rabbit intestine to see whether its ability to respond to sympathetic stimulation in the presence of ethanol or acetaldehyde was altered by pretreatment with TETD Fig 21 shows that a jejunum strip taken from a pretreated animal responded in a normal way to sympathetic stimulation in the presence of *ethanol* even at a high concentration (1 0 % w/v) The increase in tone noted on addition of ethanol to the bath was also seen in jejunum strips from un pretreated rabbits When *acetaldehyde* was present in the bath in a concentration of 0 01 % (w/v) the amplitude of the spontaneous concentrations reversibly diminished, but the inhibition following nerve stimulation was not appreciably altered (Fig 22), regardless of whether intestine strips from un pretreated or pretreated animals were used

CHAPTER VI

GENERAL DISCUSSION

The present results show first of all (Chapter III) that small ethanol doses regularly produce a fall in blood pressure and an increase in ventilation in rabbits pretreated with TETD These effects seem to correspond well to those seen in pretreated human subjects This indicates that a TETD-ethanol reaction, similar to that in man, can be produced in the rabbit

That the TETD doses which altered the response of rabbits to ethanol in the present work did not affect their general condition seems to agree with results obtained by other workers CHILD and CRUMP (1952) found an LD_{50} of 1 8 ± 0 13 g/kg for TETD in rabbits The standard dosage of TETD in the present work was considerably lower (about 0 3 g/kg for 2 days), and it was found that an altered response to ethanol could be produced also with a smaller TETD dose (about 0 15 g/kg) However, the TETD dose necessary to alter the response of man to ethanol so that a TETD ethanol reaction occurs is considerably smaller (0 01—0 02 g/kg) That no increased sensitivity to the standard anesthetic dose of urethane was seen in pretreated rabbits confirms earlier results by GRUBER (1954), who reported that the duration of action of several central nervous system depressants, including urethane, were not affected by pretreatment with TETD

Standard methods were employed in all experiments on anesthetized animals and for the *in vitro* studies The technique for recording the blood pressure from the ear artery of un-anesthetized rabbits, which permits repeated blood pressure recordings in the same animal over several days, has not been used in earlier studies of this kind, and should be of value not only in further work on TETD but perhaps also in other fields The ear artery of the rabbit derives from the superficial temporal artery which is one of the two terminal branches of the external carotid artery (BENSLEY 1946, p 286) The blood pressure level in the ear artery might therefore be slightly lower than the pressure level in the large arteries of the rabbit, but changes in systemic pressure are undoubtedly fairly accurately reflected in the ear artery pressure

As mentioned, the TETD-ethanol reaction in the rabbit has several characteristics in common with the human TETD ethanol reaction The following seem to merit particular consideration 1) In pretreated rabbits, with or without general anesthesia, ethanol doses of 1 1—4 4 mmoles/kg (i v) regularly produced a fall in blood pressure down to about 60 mm Hg and a concomitant increase in ventilation These effects appeared within 30 minutes after ad-

ministration of ethanol Similar effects of TETD + ethanol in man have been described by many workers (HALD *et al* 1948, HINE *et al* 1952, RABY 1955) The present as well as earlier results (PERMAN 1961 c) show that these ethanol doses do not appreciably affect the systemic pressure or ventilation of un-pretreated rabbits 2) *The TETD ethanol reaction* in the rabbit was, as a rule, transient with a duration (3—4 hours) similar to that of the human TETD ethanol reaction (HALD *et al* 1948) 3) In the rabbit the time between administration of TETD and the appearance and disappearance of an altered response to ethanol corresponded reasonably well to that reported in man HALD *et al* (1948) found in man that a slightly altered response to ethanol could appear about 3 hours after intake of TETD, but that full effects were not seen until 6—12 hours after intake, and that the effect of a single TETD dose lasted 3—8 days It is generally held that the protracted action of TETD is due to slow elimination (ELDJARN 1950) 4) One pretreated rabbit reacted to as little as 0 28 mmole/kg (13 mg/kg) ethanol This shows that TETD can induce a very marked supersensitivity to ethanol' in the rabbit This agrees with the observation that pretreated human subjects can experience symptoms characteristic of the TETD ethanol reaction after extremely small amounts of ethanol, for instance inhalation of ethanol vapor following application of after shave lotion (MERCURIO 1952)

In view of the consistency of the results obtained with TETD + ethanol in the present work it is difficult to explain why LARSEN (1948) did not note any clearcut circulatory changes in at least those of his rabbit experiments where a high TETD dose was used His animals (body weight 2 kg) were pretreated with 0 25—1 0 g TETD (by stomach tube) twice, 27 and 3 hours before experiments During urethane anesthesia 11—22 mmoles/kg ethanol (as a 5 per cent dilution in TYRODE solution) was infused into the jugular vein The corresponding control experiments were performed on un-pretreated rabbits He saw, as mentioned, a ventilatory increase in pretreated animals in some instances but observed no effect of ethanol on the blood pressure or heart rate in either group ZIEGLER and MEYER (1959) did not observe any TETD ethanol reaction in cats However, recent experiments by the present author have shown that pretreatment with TETD (1 g by stomach tube 27 hours before exp) can alter the response of pentobarbital anesthetized cats to ethanol (4 4 mmoles/kg i v) so that a reaction, similar to that seen in the rabbit, is produced In the cat, however, the blood pressure and ventilation changes are of smaller magnitude, which may be the reason why such manifestations were not noted by ZIEGLER and MEYER (1959) CHILD (1951) and SEIBERT *et al* (1952) did not note any sensitization" to ethanol in dogs pretreated with TETD CHILD (1951) gave no details of his experimental procedure SEIBERT *et al* (1952) pretreated their dogs with 0 01 g/kg orally for 3 days or with 0 01 g/kg intravenously (suspended in propylene glycol) half an hour before experiments It seems probable that this TETD dosage

was insufficient to produce an altered response to ethanol, since similar TETD doses (0 03 g/kg for 2 days) failed to alter the response of rabbits to ethanol in the present work

In man the characteristic TETD ethanol reaction is regularly produced by ethanol in a low dose range (< 11 mmoles/kg), and it should be emphasized that the TETD ethanol reaction in the rabbit also was produced with ethanol even in low doses *Some of the earlier results obtained in animals with TETD* and higher ethanol doses (33—110 mmoles/kg) seem to offer inferential evidence that TETD ethanol reactions do occur in animals The increased toxicity of ethanol noted in pretreated rabbits (LARSEN 1948, LECOQ 1949) and rats (CHILD *et al* 1952) could well be a consequence of a TETD ethanol reaction The fall in oxygen uptake noted in pretreated, un anesthetized rabbits (CZYZYK 1952) and mice (STAUB 1955) given ethanol could be a consequence of the circulatory manifestations of a TETD ethanol reaction Experimental evidence in favor of this supposition has been obtained by the present author in pretreated anesthetized rabbits Ethanol (4 4 mmoles/kg i v) produced first a moderate short lasting increase in oxygen uptake, but subsequently the oxygen uptake decreased well below the initial level, and this decrease coincided with the fall in blood pressure The initial increase in oxygen uptake was of an order of magnitude similar to that reported in un-pretreated rabbits (PERMAN 1961 c)

The circulatory changes underlying the arterial hypotension during the TETD *ethanol reaction in the rabbit were studied in more detail (Chapter V)* in view of the clinical importance of the corresponding hypotension in man The main implications of the present results can be summarized as follows

A transitory decline in vascular resistance in the hind limb was clearly shown, and it seems reasonable to assume that a decrease in vascular resistance occurs also in other peripheral vascular areas A decline in the total peripheral resistance therefore seems to be a major contributing cause of the hypotension, if not the primary cause In man the marked fall in diastolic pressure during the TETD ethanol reaction (Fig 1) (HALD *et al* 1948, HINE *et al* 1952) suggests a similar mechanism Transitory electrocardiographic changes are regularly seen during the human TETD ethanol reaction (RABY 1955, p 32—35), but no signs of a primary impairment of the performance of the heart were seen in the present work It seems conceivable, however, that there is a secondary impairment *of the heart function during the TETD ethanol reaction* Such factors as a diminished venous return due to peripheral pooling of blood (FERGUSON 1956) could play a role Loss of blood did not occur in the present *experiments* but the possible role of other alterations in blood volume as a contributing cause of the hypotension has not been evaluated

The decrease in peripheral vascular resistance is apparently not secondary to the increased ventilation, and results obtained in rabbits treated with atropine suggest that cholinergic vasodilation is not involved The observation

that a decrease in the vascular resistance of the hind limb occurred also after section of its sympathetic nerve supply, and that this decrease "lagged behind" the decrease in systemic pressure suggests that the peripheral vasodilation is due to a substance which appears in the blood after TETD + ethanol Further work is needed, however, to evaluate the role of production or release of vasodilating substances The failure of antihistaminics to block the TETD-ethanol reaction in the rabbit indicates that circulating histamine plays no major role Furthermore, the circulation of the rabbit is known to be comparatively resistant to histamine (*cf* PATON 1957)

Some earlier workers have proposed that the hypotension during the TETD-ethanol reaction is caused by a failure of normal homeostatic mechanisms for maintaining the blood pressure, due to impaired function of sympathetic effector mechanisms (CHRISTENSEN 1951, ROMANO *et al* 1954) However, ethanol induced a marked fall in blood pressure in pretreated rabbits also after ganglionic blockade It could also be shown in the present work that there is no absolute impairment of the vasoconstrictor system, since stimuli acting at various levels of this system (carotid occlusion, hypoxia, hypercapnia, postganglionic stimulation, administration of sympathetic transmitter) all produced the expected response There may be some impairment of the central control of the systemic blood pressure during the TETD-ethanol reaction in the rabbit It should be remembered, however, that even in the normal rabbit the homeostatic regulation of the systemic pressure is notoriously poor (*cf* KRAMER 1962), and under general anesthesia this homeostasis may have been even more seriously impaired, as suggested by fatalities among anesthetized rabbits given TETD + ethanol In man there are signs of compensatory circulatory adjustments (tachycardia, secondary pallor) during TETD-ethanol reactions, particularly when the hypotension is pronounced (HINE *et al* 1952) In the further, more detailed experimental analysis of the circulatory changes during the TETD ethanol reaction it therefore seems advisable to use animals where circulatory patterns are better known, and more similar to those of man, for instance cats

The present experiments may be considered from the point of view of ethanol and acetaldehyde metabolism Several results support the postulate that acetaldehyde is intimately involved in the TETD ethanol reaction In rabbits the maximal effect of a single TETD dose (1 g) on acetaldehyde metabolism (as indicated by the degree of acetaldehyde accumulation in the blood following ethanol) is seen after 12—18 hours, and the effect declines during the next 60—70 hours (HALD *et al* 1949 a) This time course corresponds well to that during which the response to ethanol of pretreated rabbits was found to be altered in the present work It may be concluded from earlier work (HALD *et al* 1949 a) that TETD in the present dosage does not affect the conversion of ethanol to acetaldehyde in the rabbit Earlier studies of blood ethanol disappearance rates (following i v administration) in the rabbit

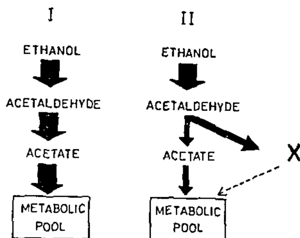


Fig 23 Schematic representation of the pathway for normal ethanol metabolism (I) and the hypothetical pathway for ethanol metabolism after pretreatment with TETD (II) X denotes the metabolite which elicits the decrease in peripheral vascular resistance

(see ELBEL and SCHLEYER 1956, p 60) show that the theoretical maximal rate at which acetaldehyde can be formed corresponds to 0 06—0 09 mmole/kg/min The observed depressor effect of acetaldehyde infusions at lower rates (0 03—0 06 mmole/kg/min) in pretreated rabbits (Chapter IV) suggests that endogenously formed acetaldehyde may be involved in the production of the hypotension during the TETD ethanol reaction

As suggested by LARSEN (1948), the ventilatory increase seen during the TETD-ethanol reaction in the rabbit could well be due to stimulation of the respiration by acetaldehyde, acting on the chemoreceptors (HANDOVSKY 1934) The slight, initial increase in arterial pH noted in the present work was probably secondary to the ventilatory increase A corresponding increase in pH has been reported in man (RABY 1955, p 28—31) The standard ethanol dose did not appreciably affect the ventilation of un-pretreated rabbits, and was therefore apparently too low to elicit the stimulation of the respiration, mainly via the chemoreceptors, which can be produced by ethanol alone (GERNANDT 1943)

However, from the present data it seems doubtful that the fall in blood pressure during the TETD ethanol reaction is due to acetaldehyde *per se* The earlier finding that acetaldehyde, when injected intravenously, has sympathomimetic properties could be confirmed in the rabbit, and this action was apparently unaffected by pretreatment with TETD The sympathomimetic effect was, however, not prominent when acetaldehyde was infused at a slow rate, a mode of administration which should more closely mimic its endogenous production from ethanol When acetaldehyde was slowly infused into un-pretreated rabbits, producing a ventilatory increase similar to that seen during the TETD ethanol reaction, there was no concomitant blood pressure fall Furthermore, a blood pressure fall with only a slight concomitant ventilatory increase was noted in a pretreated rabbit given a small ethanol dose (13 mg/kg) These observations seem to provide experimental support for the sug-

gestion made earlier (RABY 1955, p 45, and others) that the hypotension is due not to acetaldehyde *per se* but to some other substance This substance could be a metabolite of acetaldehyde and production (or accumulation) of such a metabolite (Fig 23) could well be a consequence of the inhibitory action of TETD on the normal pathways of acetaldehyde metabolism (cf HUNTER and LOWRY 1956)

Assuming that the mechanism of the TETD ethanol reaction in animals corresponds to that in man the present results open the way for a better experimental evaluation of the various counter measures which have been recommended to alleviate the sometimes serious circulatory complications during the human TETD ethanol reaction and such work is in progress It has been reported (JACOBSEN 1958) that in man promethazine or chlorpromazine seems to abolish at least the malaise during the TETD ethanol reaction, but these drugs do not block or modify overtly the reaction in the rabbit On the other hand the present findings indicate that placing the subject recumbent with elevated legs and infusing noradrenaline recommended by JACOBSEN (1958), may be an adequate supportive therapy Rebreathing into an oxygen filled bag merits trial as an emergency measure, in view of the good pressor response to hypercapnia

SUMMARY

It is well known that a small amount of ethyl alcohol (ethanol) produces a characteristic, unpleasant reaction in human subjects pretreated with tetraethylthiuramdisulfide (TETD, disulfiram, ANTABUSE). However, several workers have found it difficult or impossible to demonstrate a similarly altered response to ethanol in animals. This has made the experimental analysis of the TETD-ethanol reaction difficult.

In the present work clearcut, long-lasting hypotension and hyperventilation were regularly produced by 0.05–0.2 g/kg ethanol (i.v.) in anesthetized rabbits pretreated with TETD *(p.o.)*. One animal reacted to as little as 13 mg/kg ethanol.

A technique permitting repeated blood pressure recordings in unanesthetized rabbits was developed. With this technique it was shown that ethanol caused hypotension in pretreated animals also in the absence of general anesthesia and that the altered "sensitivity" to ethanol persisted for several days.

The TETD-ethanol reaction in the rabbit corresponds well to the human reaction in several important features.

The mechanism of the hypotension caused by TETD + ethanol was studied in anesthetized rabbits.

The hypotension was not prevented by maintaining the animal on constant ventilation (after neuromuscular blockade), nor by previous administration of atropine, mepyramine, promethazine or chlorpromazine. This indicates that hyperventilation, cholinergic vasodilation and circulating histamine are of no major importance for the hypotension.

Only a slight increase in heart rate and no change in rhythm were noted during the hypotension. There was a slight initial increase in arterial pH.

During the hypotension stimuli acting at different levels of the sympathetic vasoconstrictor system (carotid occlusion, hypoxia, hypercapnia, administration of sympathetic transmitter) all produced a rise in systemic pressure. Ethanol also induced a marked hypotension in pretreated rabbits after ganglionic blockade. These results suggest that the hypotension is not primarily due to impaired function of the sympathetic vasoconstrictor system.

The vascular resistance in the hind limb was studied *in situ* with a perfusion technique. A decrease in vascular resistance occurred during the hypotension. A slight decrease was also noted in experiments where the sympathetic nerves

to the perfused region had been cut The results suggest that a decrease in peripheral resistance is a major contributing cause of the hypotension, if not the primary cause, and that this decrease is due to a blood-borne substance which acts on the peripheral vessels

Intestines from pretreated rabbits responded in a normal way to sympathetic nerve stimulation *in vitro* in the presence of ethanol or acetaldehyde

During infusions of acetaldehyde in small amounts a ventilatory increase was observed and in pretreated animals also a blood pressure fall Rapid injection of small acetaldehyde doses caused a brief initial increase in vascular resistance (and systemic pressure) both in pretreated and in un pretreated animals These and other findings do not contradict the earlier postulate that acetaldehyde is involved in the production of the TETD ethanol reaction It seems doubtful, however, that the hypotension is due to acetaldehyde *per se* The hypotension might be elicited by a metabolite of acetaldehyde, produced or accumulated as a consequence of the inhibitory action of TETD on acetaldehyde metabolism

Clinical implications are discussed The results confirm that noradrenaline administration may be an adequate supportive therapy for the serious hypotension which may occur during TETD ethanol reactions in man

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CHANGES IN THE STATIC ELASTANCE AND HYSTERESIS OF THE CHEST WALL AND LUNGS IN NORMO-AND HYPOTHERMIA

AN EXPERIMENTAL STUDY ON CATS

BY

MATTI VAPAAVUORI

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FROM THE DEPARTMENT OF PHYSIOLOGY, UNIVERSITY OF TURKU, TURKU, FINLAND
(HEAD: PROFESSOR KAARLO HARTIALA, M.D.)

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Turku April 1962

Matti Vapaavuori

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I. INTRODUCTION

Human respiratory function consists of the exchange of gas between the atmosphere and the pulmonary capillary bed and gas exchange and utilization at the tissue level The primary object of respiration is to supply the alveolar air and consequently the blood and tissues, with oxygen and to eliminate carbon dioxide The exchange of gas between the atmosphere and the pulmonary capillary bed can be divided into ventilation diffusion and perfusion The efficiency of ventilation depends decisively on the mechanical properties of the lungs and the chest wall

The elastic properties of the lungs and chest wall have interested physiologists for over a century Carson (1820) was probably the first to study the elasticity of the lungs The principal features of respiratory mechanics were described by Donders in 1849 He was the first to point out that lungs collapse when the chest wall is opened It was not until some 70 years later that a unifying and quantitative approach to the quantities of respiratory mechanics was introduced by Rohrer (1915, 1916) Wirz (1923) and Neergaard and Wirz (1927a and b) applied it in experimental work

Inspiration is an active process The chest cage expands The pleural pressure is reduced The alveolar pressure is lower than the ambient atmospheric pressure Air enters the lungs The greater the resistance to be overcome the greater must be the pressure difference for the same quantity of air to flow into the lungs

When the lungs expand they stretch They then have potential energy, which is released during expiration This elasticity of the lungs is the first factor resisting inspiration The second factor resisting inspiration is the resistance of the airflow and the friction caused by the interacting motion of the tissues The inertia characteristics of air and each tissue is the third factor It is possible by measuring the transpulmonary pressure (the pressure difference between the pressure in the mouth and the pleural space) to determine the variation in the magnitude of this total resistance as the pressure difference during inspiration If the airways are in connection with the atmosphere through a pneumotachograph ventilator etc,

the above mentioned pressure variations due to the lungs or the chest wall must also be measured as the transpulmonary pressure

When the airflow ceases the effect of motion and inertia on the pleural pressure loses its significance The pleural pressure now illustrates merely the elastic resistance The lung elasticity is by no means a univocal concept Radford (1957) divided it into five categories

1 Elastic fibres, collagen, and reticulum of lungs, pleura, bronchi and blood vessels
2 Surface tension of gas liquid interface
3 Smooth muscle of bronchi and lungs
4 Lung blood volume
5 Elastic behaviour of bronchial mucus

The compliance ($\Delta V/\Delta P$) of the respiratory system the change in volume which is produced by the application of pressure, defines its elastic resistance to distension The pressure change is measured under static conditions when there is no air flowing The inverse of compliance, ($\Delta P/\Delta V$), *is called elastance Its dimension in this work is cm water/ml* When structural units under consideration are in parallel (*e g* , adjacent alveoli), their compliances are added to obtain total respiratory compliance When structural units are in series (*e g* , the lungs and chest wall), their elastances are added to give total respiratory elastance (Miller, Johnson and Cushing 1960)

Potential energy is stored in the lungs during inspiration and it is *released in expiration The process is not as simple for the chest wall* If the pleural pressure is made atmospheric by opening the chest wall, the lungs will retract and the chest wall expand to its resting position The resting position of the chest wall is not the position held at the resting expiratory level, but the level of c 70 per cent of vital capacity (Comroe *et al* 1956, p 115) The chest wall first promotes inspiration *in the form* of potential energy stored during expiration, until it reaches its resting *position The work done* by the respiratory muscles there upon partly becomes potential energy of the chest wall This potential energy again is released during the initial phase of expiration Expiration, unless actively assisted, is chiefly performed by the potential energy stored in the lungs However, when ventilation is more vigorous it is also performed by the potential energy *stored in the chest* wall A part of this energy is consumed by the expiration resisting action of the chest wall Expiration

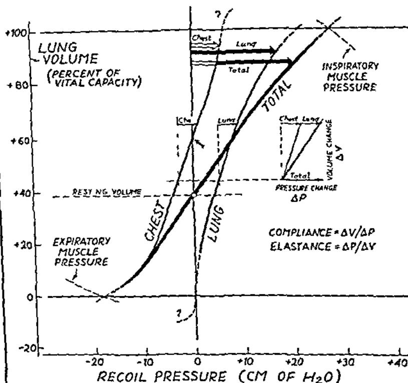


Fig 1 — Typical static pressure volume diagram

Positive recoil pressure on the horizontal axis corresponds also to negative pleural pressure for the lungs and positive pleural pressure for the chest The arrow shows the resting position of the chest wall

ends when the opposing forces of the lungs and the chest wall are balanced If expiration is aided the emptying of the lungs can be continued from this resting expiratory level to the maximal expiratory level, but with residual air in the lungs

If the respiratory muscles are relaxed by means of muscle relaxants the work of these muscles can be substituted *e g* by the overpressure of a flow generator (If the pattern of flow into the lungs, and hence of volume there is entirely determined by the ventilator and the pattern of pressure, on the other hand is the result of the effect of this flow pattern on the physical characteristics of the particular patient's lungs this type of

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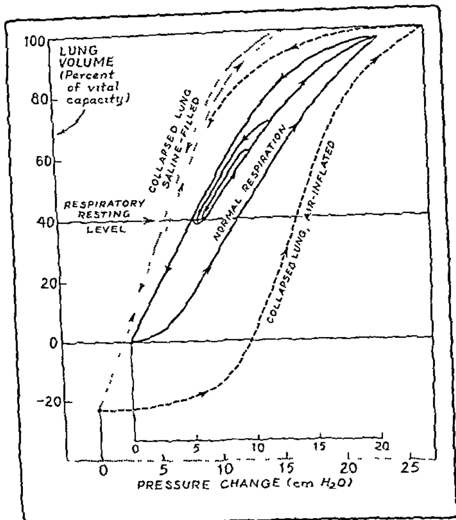


Fig 2 — Surface tension and static pressure volume hysteresis in the lungs The solid lines describe typical hysteresis of static P V curves for normal human subjects breathing at different tidal volumes As tidal volume increases, the hysteresis loop widens Hysteresis is most apparent when previously degassed dog lungs are inflated with air (heavy dashed line) and it is almost completely eliminated when air fluid interfaces are absent during inflation of the same dog lungs with saline (dotted lines)

ventilator is called a flow generator — Mapleson 1959 p 43) The changing of the pleural pressure thus illustrates the fluctuations in the elasticity, motion and inertia of the chest wall and if the airflow ceases it illustrates merely the elastic resistance of the chest wall (The chest wall in this work refers to all the parts surrounding the lungs which participate in one way or another in respiratory function such as the chest wall itself diaphragm abdominal muscles etc)

Fig 1 presents the static pressure volume curve It is also called a relaxation pressure curve It can be measured in two different ways 1 The lungs of a subject relaxed by means of muscle relaxants are inflated or deflated by changing the respirator stroke volume by degrees and measuring the pressures for different volumes 2 The subject inspires or expires a certain quantity of air of his own volition He then relaxes his nostrils closed and one end of the water manometer in his mouth The pressures for different volumes are measured It is essential that the vocal cords are open

It has now been established that the position of this static curve varies during the respiratory cycle (Dean and Visscher 1941 Radford Lefcoe and Mead 1954 Butler 1957) Input pressures during inspiration are higher than output pressures measured at the corresponding volume levels *during expiration This phenomenon is called static hysteresis (Fig 2)* The terms hysteresis and hysteresis loop are defined by Landowne and Stacy (1957) Hysteresis is the failure of a system to follow identical paths of response upon application of and withdrawal of a forcing agent The result of this failure to retrace the same path on withdrawal as on application is the formation of a hysteresis loop

The present opinion is that the surface tension acting at the liquid air interface within the lungs has a major role in static hysteresis Some investigators however advocate the role of the plasticity of the lung tissue

Bull (1951 p 192) states The static surface tension is the equilibrium tension the dynamic tension is the tension of a liquid before the surface film has had time to form The time required for the attainment of surface equilibrium of pure liquids is exceedingly small probably of the order of a millionth of a second The time required for a solution in which the solute molecules are of moderate size to reach equilibrium is also exceedingly short It is only in dealing with solutions of colloidal materials that the time required for the formation of the surface is appreciable The surface tension of solutions of colloidal surface active substances present several complicated features For one thing the time required

II. OBJECTS OF THE PRESENT INVESTIGATION

The changes in the position of the static pressure volume curve during the respiratory cycle are continuously of importance in investigations of *respiratory mechanics* Most of the modern studies emphasize the surface tension of the lungs as an agent of static hysteresis although there are advocates still who stress the plasticity of the lungs in this process There is repeated reference in connection with surface tension to the difference in the number of open alveoli at the corresponding volume level in inspiration and expiration On the other hand the stabilizing effect of area surface tension hysteresis on alveoli during expiration reported by Clements and Brown raises the question of the relative part played by area surface tension hysteresis in static hysteresis compared with the effect caused by the difference in the number of open alveoli Mead (1961 p 297) writes on the importance of differentiating between these factors The distinction is important since the implication of the two mechanisms on the capillary surface area exposed to gas and on the relative distribution of ventilation and perfusion is altogether different This differentiation is fundamental to an understanding of gas exchanges in the lungs and it is a major unsolved problem of pulmonary mechanics'

Despite the present voluminous literature on hypothermia its effect on respiratory mechanics is hardly known at all For this reason the effect of hypothermia on static hysteresis and elastance of the chest wall and lungs has been investigated in the present study

The objects of the present work are to establish

1 the relation of static hysteresis caused by the difference in the number of open alveoli to static total hysteresis
2 the effect of hypothermia on static hysteresis
3 the variations of the static elastance of the lungs and chest wall when the temperature is lowered from 38°—36°C to 10°—8°C
4 whether the leakage of air from the lungs by the alveolar route or otherwise during measurement is a factor distorting the results

for attainment of equilibrium in many substances is very long, and it is really doubtful that in some a true equilibrium tension is ever observed"

The opening and closing pressure of alveoli are proportional to the surface tension prevailing in them The investigators (e g Mead, Whittenberger and Radford 1957 and Radford 1957) who emphasize the role of surface tension as the causative factor of static hysteresis claim that static hysteresis is caused by the difference in the number of open alveoli which occurs at the corresponding volume levels during inspiration and expiration

In their recent studies with lung extract, Brown, Johnson and Clements (1959) established that the surface tension is greater when the film is expanded than when it is compressed They concluded from this that this so called area surface tension hysteresis has a stabilizing effect on the alveoli The alveoli would therefore tend to remain open during expiration

There are many studies about the properties of respiratory mechanics in normothermia They reveal that a wide variety of diseases may alter the balance of forces affecting ventilation Weakness of respiratory muscles disturbances of the thoracic structure and recoil, disorders of the pleural space and disorders of mechanical response within the lungs have been noted to lead to a change in the respiratory mechanics relations Circulatory factors also may affect them The effect of postural changes, assisted or controlled respiration, the depth of anesthesia, etc on the properties of respiratory mechanics have also been studied The plasticity of the lung tissues and physico chemical properties of the liquid in the alveoli have been of focal interest in these investigations By contrast, little attention has been paid to the changes possibly caused by hypothermia

It has been possible to identify the most important factors contributing to these phenomena The relative contribution of these factors to the total event, however, is a matter of considerable differences of opinion

III. REVIEW OF THE LITERATURE

A ELASTANCE (COMPLIANCE)

1 General

Bayliss and Robertson (1939) called the pressure-volume ratio ($\Delta P/\Delta V$) elastance Mead and Whittenberger (1953), again, used the inverse of elastance which they called compliance

Carson (1820) was the first to measure the elastic tension of the lungs. The first in vivo measurements of the volume-pressure ratio were performed by Neergaard and Wirz (1927 a) They made their observations on two patients one with tuberculosis and the other with emphysema Ferris *et al* (1952) were probably the first to measure the chest wall factor by subtraction of the total respiratory compliance and lung compliance values.

In Finland Jalavisto (1955), Haaranen *et al* (1957) and Larmi and Appelqvist (1961 a and b) have studied the retractive force of the lungs

2 *Relation between esophageal and pleural pressure*

In the earlier studies of respiratory mechanics the variations in the pleural pressure were registered by puncturing the pleural space This method was employed *e g* by Neergaard and Wirz (1927 a), Christie and McIntosh (1934) Paine (1940) and Dayman (1951) Since the pleural puncture involves special risks its use is limited in investigations of man

Buytendijk (1949) substituted a method of measuring the pleural pressure by taking the esophageal pressure He found that the values correspond with sufficient accuracy Good agreement between pleural and esophageal pressure variations was established also *e g* by Fry *et al* (1952) and Dornhorst and Leathart (1952)

On the contrary Butler White and Arnott (1957) by comparing the compliance values for pleural and esophageal pressures in human subjects found a somewhat higher compliance for esophageal pressures during spontaneous breathing This was also shown *e g* by Mead and Gaensler (1959) They noted that the individual changes in the esophageal pressure deviated from the corresponding changes in the pleural pressure measured in the upright posture (20 to —18 per cent) Supine the respiratory fluctuations not only deviated still more (60 to —25 per cent) but were consistently greater a factor tending to give falsely low values for lung compliance

It has been found by Farhi Otis and Proctor (1957) that the pleural pressure measured simultaneously at various points over the surface of

The last problem is closely associated with basic research In the present writer s opinion, the literature does not devote sufficient attention to this question The study of the leakage of air from the lungs was added to the problems to be investigated as it is generally of significance in the experiments of hysteresis and elastance and it is also essentially associated with the interpretation of the results

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It has been found by Farhi, Otis and Proctor (1957) that the pleural pressure measured simultaneously at various points over the surface of

lungs in dogs was essentially identical except at the apices and in the *diaphragmatic sinus Coleridge and Linden (1954)* noted that the pressure in the lateral pleural space was more negative than in the medial one This difference varied from three cm water during quiet breathing to eight cm water at deep inspiration

It is possible to measure the static pleural or esophageal pressures for different volume levels by instructing the subject to hold his breath at various intervals (Rahn *et al* 1946) Static pressures can also be measured by interrupting the airflow in the airways at regular intervals with a technical arrangement of valves (Stead, Fry and Ebert 1952, Brown, Fry and Ebert 1954, Frank *et al* 1956) Results corresponding to satisfactory static values were obtained by Buytendijk (1949) by making the subject breathe as slowly as possible The same method was used by Butler, White and Arnott (1957) and Ehrner (1960)

3 The effect of anesthesia and relaxants on the elastance

Rahn *et al* (1946) assumed that the subject is capable of complete relaxation when his relaxation pressure-volume curve is measured Nims, *Conner and Comroe (1955) studied the validity of this assumption They* measured the total respiratory compliance in anesthetized subjects after hyperventilation and after the administration of muscle relaxants These values were compared with results obtained by the relaxation method before anesthesia and with the values given by Rahn *et al* (1946) for conscious subjects The comparison showed that even allowing for the potential error of the method the compliance values for conscious subjects were falsely high They were not capable of complete relaxation when inspiring

Lung compliance was measured by Howell and Peckett (1957) in the same subject before and after the induction of anesthesia and muscular paralysis Compliance fell by an average of 30 per cent in investigations *performed on four subjects* They noted that the chest wall compliance decreased, too It seemed to them, however, the major factor contributing to the decreased compliance under anesthesia appeared to be a decrease in lung compliance Foster, Heaf and Semple (1957), on the contrary, found no changes in five subjects of their series of ten The lung compliance rose in four cases and fell in only one case Wu, Miller and Luhn (1956) noted that lung compliance decreased during two hours after the induction of anesthesia by on average of 35 per cent in human subjects breathing spontaneously in the supine position *Holaday and Israel (1955) and*

Brownlee and Albritten (1956) measured the total respiratory compliance during intermittent positive pressure breathing (I P P B) and anesthesia They noted that compliance fell They did not describe the time course of the changes

Pentobarbital anesthetized apneic dogs were studied by Safar and Bachman (1956) They observed that total respiratory compliance remained unchanged when the depth of anesthesia varied provided that intermittent positive pressure breathing was constant. Spontaneous respiration lowered the compliance The variations of the total respiratory compliance were studied by Safar and Aguto Lscarraga (1959) in 54 anesthetized apneic adults They found that compliance varied in the same subject according to the degree of muscle relaxation the type of breathing (I P P B , spontaneous respiration etc) and changes of positions on the operating table The variations in the depth of cyclopropane anesthesia did not change the compliance values during a steady state of I P P B provided that the subject did not cough strain or breathe Spontaneous breathing on the other hand lowered the compliance They ascribed this to minute atelectatic areas hypostatic congestion of the lungs or the synergetic effect of both. Hyperinflation raised the compliance values to the control level or even higher

It was established by Mead and Collier (1959) that the lung compliance was at its maximum immediately after forced inflation and at its minimum following forced deflation After forced inflation compliance in a course of time was reduced rapidly whereas it remained unchanged after forced deflation. With more vigorous inflation compliance always returned to near maximal levels Drinker and Hardenbergh (1948) noted the ab normal appearance of lungs of dogs kept under deep anesthesia and held supine for some hours The lower lobes dorsally and extending variable distances towards the apices were congested and liverlike in appearance They let anesthetized dogs in a supine position breathe aerosol containing Evans blue (T 1824) On autopsy the dye was demonstrable only in the anterior parts of the lungs Mead and Collier (1959) made the same observation They presented a series of pictures showing that the irregular principally dorsal and caudal dark areas of lungs established on autopsy can be eliminated by inflation

Landmesser (1947) found that d tubocurarine caused bronchoconstriction in 80 per cent of the dogs studied Landmesser Converse and Harmel (1952) noted on the contrary that it did not cause bronchoconstriction in human subjects except one D tubocurarine lowers total respiratory

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5 The effect of hypothermia on the elastance

There are in the literature very few studies concerning the effect of hypothermia on elastance and other respiratory mechanics Hypothermia depresses markedly pulmonary ventilation (Blair 1960) The magnitude of this depression and the temperature of its endpoint apnea, depend greatly on the depth of anesthesia and individual variations (Severinghaus 1959) Anatomical dead space studied by Severinghaus and Stupfel (1956) increased 70—90 per cent when the temperature was lowered down to 26°—22°C They observed that physiological dead space increased, too but that alveolar dead space remained unchanged during hypothermia.

Bayliss and Robertson (1939) obtained that elastance increased if excised lungs were allowed to cool but not if they were warm Severinghaus and Stupfel (1955) noted that compliance diminished with the temperature but that the changes were not significantly greater than those occurring within corresponding time at a temperature of 37°C They regarded the primary cause of the progressive reduction in compliance as atelectasis and congestion more rarely as edema Sechzer (1958) found no significant change in the total respiratory compliance or resistance when the body temperature was reduced to as low as 29°C Mead (1961, p 299) notes in his review a personal communication with Radford and Frank There is very little change in lung retraction with temperature between 20°C and 37°C The pressure-volume curve of the pulmonary vascular bed in the dog studied by Sarnoff and Berglund (1952) using an isolated lung preparation did not change when the perfusion blood was cooled from 39 4°C to 28 9°C

Since organic colloidal solutions are mostly hydrophilic sols they have a low surface tension and a high viscosity (Tommila 1961 p 491) The viscosity and surface tension of both pure liquids and colloidal solutions increase when the temperature is lowered Seifritz (1952) established a considerable rise in viscosity of protein and protoplasm between 38°C and 2°C The surface tension of water at 37°C is about 70 dyn cm ¹ and that of blood serum about 47 dyn cm ¹ The above values are at 17°C about 73 dyn cm ¹ and 57 dyn cm ¹ respectively (Geigy 1960)

Van t Hoff s rule states that most chemical processes will double or treble with a 10°C increase in temperature A temperature coefficient per 10°C (Q_{10}) ranges from 1 4 to 2 0 for enzymic reactions and from 2 to 4 for most chemical processes On the contrary the above coefficient is only 1 2—1.3 for physical processes (Rauen 1956 p 690)

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4 *Changes of elastance due to posture and variations of functional residual capacity*

In a careful study of respiratory mechanics in the sitting and supine positions of normal subjects, Attinger, Monroe and Segal (1956) found that lung compliance was distinctly lower in the supine position Great total respiratory compliance variability according to posture was found by Safar and Aguto Escarraga (1959) The investigations were performed on relaxed, anesthetized human subjects in different operating positions *For instance, the average total respiratory compliance fell to 82 per cent* when measured in the Trendelenburg position (head down 20 degrees), whereas the 20 degrees head up position increased the average compliance to 118 per cent of that in the supine position Several investigators, *e g* Ferris Mead and Frank (1959) have confirmed that compliance changes are produced by alterations in position They noted that in the supine position false values of esophageal pressure and lung compliance may be obtained Further they stated that measurements of esophageal pressure and lung compliance in recumbent subjects are most reliable when made in the prone or lateral positions Lim and Luft (1959) found however that the so called specific compliance ($\Delta V/\Delta P/FRC$) in their subjects obtained in the erect standing and supine positions showed no significant difference in the presence of marked alterations in non specific compliance and functional residual capacity (FRC)

The functional residual capacity is greater in the recumbent position than in the upright (Kory and Smith *1960, p 551) Butler (1957) observed* that the lungs and chest wall *of adults* upon passive deflation, *do* not return immediately to their *initial resting volume levels after inflation* to *20 cm water pressure* A return to resting values occurs after about ten seconds

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There are in the literature very few studies concerning the effect of hypothermia on elastance and other respiratory mechanics Hypothermia depresses markedly pulmonary ventilation (Blair 1960) The magnitude of this depression and the temperature of its endpoint, apnea depend greatly on the depth of anesthesia and individual variations (Severinghaus 1959) Anatomical dead space studied by Severinghaus and Stupfel (1956) increased 70—90 per cent when the temperature was lowered down to 26°—22°C They observed that physiological dead space increased, too, but that alveolar dead space remained unchanged during hypothermia.

Bayliss and Robertson (1939) obtained that elastance increased if excised lungs were allowed to cool but not if they were warm Severinghaus and Stupfel (1955) noted that compliance diminished with the temperature but that the changes were not significantly greater than those occurring within corresponding time at a temperature of 37°C They regarded the primary cause of the progressive reduction in compliance as atelectasis and congestion more rarely as edema Sechzer (1958) found no significant change in the total respiratory compliance or resistance when the body temperature was reduced to as low as 29°C Mead (1961, p 299) notes in his review a personal communication with Radford and Frank "There is very little change in lung retraction with temperature between 20°C and 37°C The pressure-volume curve of the pulmonary vascular bed in the dog studied by Sarnoff and Berglund (1952) using an isolated lung preparation did not change when the perfusion blood was cooled from 39 4°C to 28 9°C

Since organic colloidal solutions are mostly hydrophilic sols they have a low surface tension and a high viscosity (Tommila 1961 p 491) The viscosity and surface tension of both pure liquids and colloidal solutions increase when the temperature is lowered Seifritz (1952) established a considerable rise in viscosity of protein and protoplasm between 38°C and 27°C The surface tension of water at 37°C is about 70 dyn cm^{-1} and that of blood serum about 47 dyn cm^{-1} The above values are at 17°C about 73 dyn cm^{-1} and 57 dyn cm^{-1} respectively (Geigy 1960)

Van t Hoff s rule states that most chemical processes will double or treble with a 10°C increase in temperature A temperature coefficient per 10 C (Q_{10}) ranges from 1 4 to 2 0 for enzymic reactions and from 2 to 4 for most chemical processes On the contrary the above coefficient is only 1 2—1 3 for physical processes (Rauen 1956 p 690)

B HYSTERESIS AND STRESS RELAXATION

1 *General*

The difference between the pressure volume expiration curves in connection with filling with air and liquid was established by Neergaard as early as in 1929 He measured, however, only the expiration curve after almost maximal inflation This is why he failed to establish the hysteresis phenomenon Hirakawa demonstrated hysteresis in dead animal lungs in 1924 Dean and Visscher (1941) found greater hysteresis after death than in the lung with intact circulation Dean and Visscher (1941), using dogs, seem to have been the first investigators conclusively to demonstrate hysteresis in the living lung This static hysteresis was later investigated *e g* by McIlroy (1952), Radford, Lefcoe and Mead (1954), Butler (1957), Mead, Whittenberger and Radford (1957), Radford (1957) and Agostoni *et al* (1958) Butler studied also the pressure volume diagram of the chest wall in human subjects with a normal chest He stated that the hysteresis effect of the chest wall is probably due to the stretching of muscles and ligaments

Several observations suggest that the lungs are lined by a fluid layer with intense surface activity Terry (1926) observed free fluid in the alveoli during life Macklin (1954) stated that this material has been credited with performing vital functions, such as assisting in the removal of fine living and dead particulate matter It seemed to him that there was evidence pointing to the granular pneumonocytes as the originators of the secretion which composes this film Pattle (1955) noted an unusual stability in the form of pulmonary oedema fluid and in fluid expressed from the cut surface of fresh lung tissue He concluded that the stability of the foam was due to an insoluble surface layer of protein that arose from the lungs Brown, Johnson and Clements (1959) claimed that there is apparently on the alveolar surface a peculiar material which has a coefficient of compressibility that places it in the category of a liquid film In their opinion the evidence that the material in the film is mucoprotein is strong but not conclusive

'How are the air spaces stabilized? is a question put by Mead (1961 p 294) in his review He continues Three mechanisms have been described, two of them involve the surface and a third the tissue surrounding the surface The first is the case that Neergaard (1929)

considered where all surfaces are less than hemispheres and radii of curvature decrease as volume increases It will be referred to as geometric stability The second operates beyond the hemispheric shape and involves the change in tension with area discovered by Brown and Clements It will be referred to as surface film stability The third stabilizing influence depends on the tissues surrounding the air spaces The ratio of the parts played in static hysteresis and stress relaxation by the difference in the number of open alveoli the pulmonary tissue and the area surface tension hysteresis discovered by Brown and Clements is still unknown

2 Static pressure volume hysteresis

Bernstein (1957) studied the static pressure-volume curves of the living rabbit He noted that the first measured so called unadapted curve differed from the following adapted curves The tracheal pressure in the first curve at a given volume level was higher than the corresponding pressure of the second and following curves He concluded that this was due either to a plastic (non elastic) stretching of the lungs and possibly of the chest wall or the difference in the number of open alveoli between the unadapted and adapted curve at the corresponding volume levels

Surface tension acting at the liquid air interface within the lung has been noted to be one of the most important factors causing static lung hysteresis Although it is possible in their opinion that this behaviour reflects also the non reversible stress-strain characteristics of tissue elements within the lungs Mead Whittenberger and Radford (1957) stressed the role of surface tension They considered that much of the hysteresis of the excised airfilled lungs must relate to the irregular expansion the number of open alveoli at the corresponding volume levels is greater during expiration than during inspiration

In this connection should also be mentioned the recently published studies by Pierce Hocott and Hefley (1961) 'Any regularly shaped geometric figure has a unique ratio of surface to volume at every different size An increase in the radius of curvature leads to a decrease in this ratio In considering the entire lungs the ratio of surface to volume must reflect the mean size of the spaces in the lungs They noted however, that this ratio diminishes with the volume In their opinion this could only be interpreted as indicating that the mean radius of curvature of the air spaces increased as volume was removed from the lungs This must have resulted from the closure of some of the smaller air spaces

In Radford's experiment (1957) the lungs were first filled with saline and then inflated with air. He pointed out that 1. Much higher pressures were required to inflate the lung to a given volume with air than with saline. 2. Until a pressure greater than 8 cm water was reached, essentially no air entered the lungs (the opening pressure phenomenon) and subsequent filling with air took place usually in a very patchy fashion. 3. During deflation, although emptying was quite uniform, higher pressures with air were still required to maintain the same lung volume than with saline. The opening pressure effect was observed already by Lindskog and Bradshaw (1934) and Gruenwald (1947). Agostoni *et al.* (1958) emphasized in addition to the surface tension the significance of the viscosity of the liquid in the opening pressure phenomenon. Jaykka (1957) established the significance of "capillary erection" in this phenomenon in newborn. The significance of pulmonary circulation was confirmed *e.g.* by Peltonen and Kiemer (1961). Peltonen and Hirvonen (1960) emphasized that the negative pleural pressure is of great importance, too.

Agostoni et al. (1958), on the other hand, wrote as follows: "Another feature of the pressure-volume diagram of the fetal lung observed by introducing liquid into the lung is the presence of a hysteresis area similar to that demonstrated in the adult. In these conditions the hysteresis area cannot be accounted for by the surface tension, as it has been done by some (Mead, Whittenberger and Radford 1957) for the adult lung. The present finding confirms the view that the hysteresis is an intrinsic mechanical feature of the pulmonary tissues probably due to their plasticity. This view is also supported by the fact, already shown in the adult lung (Setnikar 1955, Agostoni and Taglietti 1957), that the slower the rate of inflation and deflation the larger the hysteresis area."

Pressure-volume properties have been studied also by Radford, Lefcoe and Mead (1954) in cat, dog and rat lungs. In their opinion static lung hysteresis may be due to properties of the bronchial smooth muscle, to opening of previously closed bronchioles or alveoli, or to other complex geometric factors. On the other hand Radford and Lefcoe (1955) found in excised fluid-filled lungs only small changes in retractive pressures following induced contraction and relaxation of the smooth muscle in the tracheobronchial tree. This conclusion is supported by Mead and Collier (1959) and Pierce, Hocott and Hefley (1961). They claimed that the bronchial and bronchiolar smooth muscle and the amount of blood in the pulmonary vascular bed are factors of minor importance in static hysteresis, although it has been shown by Borst *et al.* (1957) that in dogs

acute changes in pulmonary arterial and venous pressures are associated with changes in the mechanical behaviour of the lungs

3 Area surface tension hysteresis

Clements (1956 1957) studied the surface tension area behaviour of surface films prepared from rat cat and dog lungs His observations support the view that surface tension is a primary determinant of the static behaviour of the lungs but these data suggest however that surface tension varies with lung volume and from point to point within the lungs at a given volume He stated that this characteristic of the surface has a stabilizing influence and might be called an antiatelectasis factor He did not observe similar effects with extracts of blood plasma Brown Johnson and Clements (1959) noted that the film prepared from lung extract can apparently be reversibly compressed to 50 per cent of its initial area After further compression the film apparently ruptures on re-expansion They noted that the relative surface tensions obtained from the pressure volume curves of air and saline deflations of the lungs of rats cats and dogs were similar From the assumed 50 dyn cm⁻¹ value surface tension decreased markedly on deflation Limiting values were as low as 5 dyn cm⁻¹ Clements *et al* (1961) developed further the theory of alveolar stability caused by this area-surface tension hysteresis of film prepared from lung extract They studied surface tension area diagrams from lung extracts of adult human lung normal rat lungs premature infant s lungs and also rat lungs which were treated with a nonionic detergent (5 % Tween 20 in saline) They noted that in normal rat lungs for instance the area surface tension hysteresis was considerable whereas it disappeared in rat lungs treated with a nonionic detergent In the last mentioned case the lungs had the greatest tendency to become atelectatic

4 Stress relaxation

Butler (1957) observed that after the lungs are inflated to constant volume there is a slow fall in transpulmonary pressure and the opposite occurs after a deflation This so called stress relaxation has been noted earlier *e g* by Bayliss and Robertson (1939) and Mount (1955) Hughes May and Widdicombe (1959) studied stress relaxation in rabbit lungs both because it might offer a different method of examining hysteresis (compared with the plotting of pressure volume curves) and in an attempt

to discover why the lungs are imperfectly elastic After eliminating other possible reasons, they came to the conclusion that stress relaxation is thought to be due to a yielding of pulmonary tissue in addition to surface tension effects, and it is comparable with the stress relaxation seen in other tissues In their opinion any change in the gas content of the lungs, due to greater absorption of oxygen than output of carbon dioxide, would not be enough to produce the fall in pressure observed, even assuming that the respiratory quotient were as low as 0 7

Marshall and Widdicombe (1961) mentioned the following reasons as possible causes of stress relaxation

1 Redistribution of air in the lung whilst the breath is held,
2 Opening and closing of alveoli, a critical closing effect,
3 *Stress relaxation of the tissues of the bronchi or alveoli This could occur in the collagenous, elastic or muscular tissues,*
4 Flow of blood into the lungs relaxing the stretch on the other elements of the lungs

They concluded that stress relaxation is not due to redistribution of gas or to flow of blood into the lungs They went on to say 'Although opening of alveoli can explain the phenomenon of stress relaxation the occurrence of stress relaxation in the fibres of the lung tissue is not ruled out Stress relaxation might occur in either the muscular or connective tissues of the lung'

Stress relaxation *in the isolated trachea of rabbits has been demon*strated by Hughes, May and Widdicombe (1959) who noted that the amount of relaxation is increased when the muscle tone is increased by the addition of acetyl choline to the organ bath Stress relaxation in smooth muscle has been noted by Bozler (1941) Marshall and Widdicombe (1961) considered however, that stress relaxation of smooth muscle fibres is less likely to account for the relaxation seen in the lung for the muscle content of the lungs (excluding the bronchi) is small on the other hand the elastin content of the lung is high (Briscoe and Loring 1958) and some stress relaxation could occur in the elastic fibres

IV. THE PRESENT INVESTIGATION

A MATERIAL AND METHODS

Experiments were performed on 40 mongrel cats weighing between 1 9 and 3 0 kg (B_1 15 cats B_2 10 cats B_3 15 cats) Five of these animals made up a group of experiments to determine the effect of the time factor An additional 10 cats and 20 rabbits were used in advance to develop the method used in this work for comparative studies in normo and hypothermia The normothermia values of each subject form a control group The same animal was always used in these comparative studies to reduce to a minimum the role of potential disturbing factors The cats were anesthetized with pentobarbitone sodium intraperitoneally (40 mg/kg) and the rabbits with urethane intravenously (0 3—0 4 g/kg) Suxamethonium chloride in small repeated doses of 25—50 mg was used to relax the muscles during the experiments

When the animal had become unconscious which took c ten minutes it was placed in the supine position in a plastic basin A support was placed under its neck The subject thus assumed the optimal position for tracheostomy The limbs were fixed obliquely upward to the edges of the basin The tracheostomy was made as bloodless as possible by pre ligating the vessels likely to bleed One arm of a T-shaped metal tube was inserted into the trachea and tied firmly in place with wire The other arm of the T tube was connected with a variable stroke ideal pump F 19a (C F Palmer ltd London) A rubber tube leading to a water manometer was attached to the third arm of the T tube The rubber tube was then closed The stroke volume of the respirator was adjusted for the size of the animal 40—60 ml Suxamethonium chloride was injected intramuscularly When muscle relaxation had been achieved the chest wall was cautiously opened at the 5th intercostal space A no 8 Cournand single lumen cardiac catheter with a two centimeter long perforated end of Nelaton catheter fitting the former was selected as the pleural catheter in the preliminary experiments This catheter was inserted into the pleural space The perforated Nelaton catheter end prevented the expanding lung from plugging the open end of the cardiac catheter and thus from reducing pleural pressure at times The pneumothorax was eliminated by moment arily increasing the respirator stroke to 150 ml and by using a syringe to

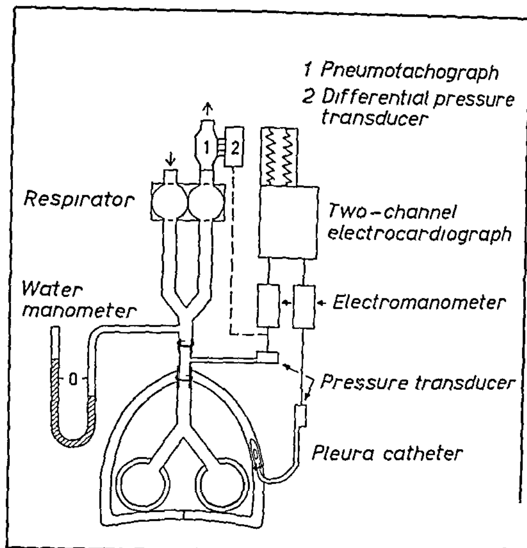


Fig 3 — The technical arrangement of the static elastance and hysteresis measurement

aspirate concurrently via the catheter When an elastic resistance had thus been achieved through suction 3 ml of air was injected into the pleural space to prevent the lung from plugging the opening of the catheter This pleural catheter was fixed to the pressure transducer (Elema Schonander Stockholm), ensuring that the pressure values were not falsely reduced Tracheal pressure was measured in a corresponding way with the aid of an injection needle at the upper level of the T tube The temperature of the rectum was monitored with an electric thermometer (Elektrolaboratoriet Kobenhavn) The ECG leads were fixed with needles to the limbs of the animal The ECG was recorded with a two channel

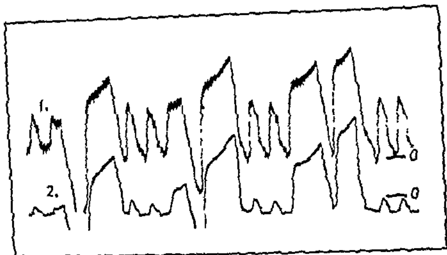


Fig 4 — Tracheal (1) and pleural (2) pressures when an unrelaxed test animal is used Stroke volume 50 ml Vertical scale 1 cm = 3 5 cm H_2O

direct writing oscillograph (Mingograf , Elema Schonander, Stockholm) A Fleisch pneumotachograph (Mivab, Stockholm) and a differential pressure transducer (Elema Schonander, Stockholm) were used to determine the instant of zero flow The pneumotachograph was placed at the mouth of the expiration tube of the respirator to record the exact moment of the start of expiration (Fig 3)

Before the animal was anesthetized, all the pressure-recording apparatuses were calibrated against a water column to detect as sensitively as possible changes in the pressures of the magnitude of a couple of centimetres of water with satisfactory accuracy The recording positions of the different curves on the paper were adjusted to correspond to one another temporally Calibration was checked at the termination of the experiment, often during it as well The stroke volume during the experiment was later checked by pneumatic measurements Static measurements were performed slowly at a respirator velocity of 5 or 12 strokes per minute The animal was autopsied after the experiment Vigorous inflation was applied to ensure that the lungs were intact Before this, the lungs were examined for atelectatic changes The airways were then opened as far as the periphery to ascertain whether mucus had played any part in the results Comparative studies in normo- and hypothermia were one of the most important reasons for choosing an animal that breathed with a respirator as the subject Fig 4 shows why the animal was relaxed

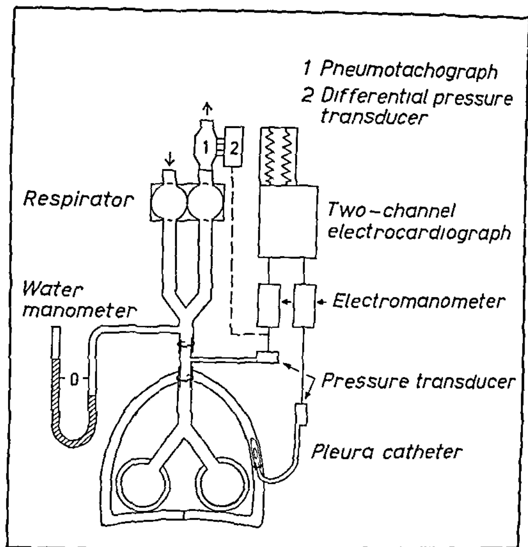


Fig 3 — The technical arrangement of the static elastance and hysteresis measurement

aspirate concurrently via the catheter When an elastic resistance had thus been achieved through suction 3 ml of air was injected into the pleural space to prevent the lung from plugging the opening of the catheter This pleural catheter was fixed to the pressure transducer (Elema Schonander, Stockholm), ensuring that the pressure values were not falsely reduced Tracheal pressure was measured in a corresponding way with the aid of an injection needle at the upper level of the T tube The temperature of the rectum was monitored with an electric thermometer (Elektrolaboratoriet København) The ECG leads were fixed with needles to the limbs of the animal The ECG was recorded with a two channel

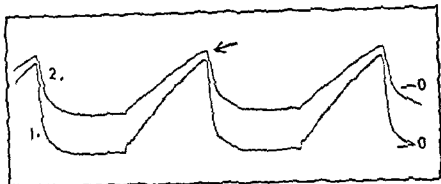


Fig 6 — The tracheal (1) and pleural (2) pressure reach the peak value at exactly the same time during static measurement
Stroke volume 100 ml Vertical scale 1 cm = 4 cm H_2O Horizontal scale 1 cm = 2 5 sec

animal during inspiration in static and near static measurements always corresponded to the instant of zero flow Concurrent recording of tracheal and pleural pressure showed likewise, that both pressures reached their peak value at exactly the same time (Fig 6) The maximum of the pleural pressure in the near static measurements also corresponded thus to the instant of zero flow The horizontal part of the pressure curves shows that a return to resting volume levels occurs before a following stroke The moment of start of inspiration on the pressure curves always corresponds to the moment of termination of the horizontal part of these curves As it was possible in this way to show accurately the instants of zero flow of the respiratory cycle only the tracheal and pleural pressures were measured in this work This was in order to avoid the errors caused by the different recording apparatuses when the pressure and pressure differences of a few centimetres of water are measured electronically and to perform an experiment with optimal co-ordination A simple water manometer (Fig 7) was employed for the same reason in a part of the experiments, either to control the results or to obtain new results. Since, as stated above, the maximum pressure is attained in the static measurements of pleural pressure and tracheal pressure at exactly the instant of zero flow, this manometer can be used for comparative studies of the maximum pressure in different conditions The respirator stroke volume can be read from the respirator Care was taken to ensure that the position of the stroke volume regulator always coincided with the volume in question

Cooling of the animal Cold water was led into the basin. When the animal was half covered with water, ice was added into the basin Special

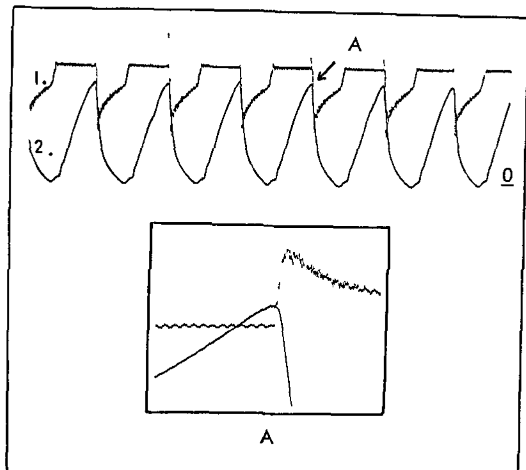


Fig 5 — The peak value of the tracheal pressure corresponds temporally to the instant of zero flow

Pneumotachogram (1) and tracheal pressure curve (2) Point A The opposite direction of the pneumotachogram is due to technical reasons Stroke volume 100 ml Vertical scale 1 cm = 4 cm H_2O Horizontal scale 1 cm = 1 5 sec

The use of an unrelaxed animal introduces unexpected additional factors which complicate the interpretation and can influence the results markedly

When the cat is the subject, the pneumotachograph between the respirator and the animal increases the dead space considerably and can even involve a risk to life The shape of the pneumotachogram tends to vary despite efforts to the contrary, for this reason in the preliminary experiments the pneumotachograph was placed at the mouth of the expiration tube of the respirator to record the moment of onset of expiration (the instant of zero flow) It was found with the aid of numerous measurements (Fig 5) that the maximum of the tracheal pressure of a relaxed

inspiration and expiration The difference in the method applied in the present work was in measuring only the maximum of the pressure during the respirator stroke the instant of zero flow The relationship between these maxima of the input and output pressures and the corresponding volumes (respirator strokes), the pressure volume curve, was measured by increasing the stroke gradually up to 200 ml and then decreasing it correspondingly The sum of the pressure differences of three volume levels (50 ml 100 ml and 150 ml) on this curve was taken as the measure of hysteresis except the measurements performed only at a single volume level (Fig 8) This procedure ensured that the leakage of air established in the preliminary experiments, especially when the stroke volume used was 100 ml or over, did not affect the results. The peak values of the tracheal pressures corresponding to the above mentioned volume levels ranged in this work

ml	from cm water	to cm. water
50	3 4	6 8
100	8 7	12 0
150	14 4	19 0
200	20 0	28 2

The measuring time of hysteresis could influence the results, likewise the position of the stroke volume regulator For this reason a part of the measurements was conducted at a single volume level only The measuring time was thus as short as possible In these cases the lungs were expanded by closing the respirator expiration tube for the duration of 3—4 strokes The pressures measured at the same volume level during inspiration and expiration are thus really comparable Butler (1957) observed that the lungs and chest wall of adults upon passive deflation do not return immediately to their initial resting volume levels A return to resting values occurs after about ten seconds In static measurements the resting expiratory level must however be regarded as constant in fully relaxed cats breathing with a respirator and whose position is unchanged during the experiment

b The effect of hypothermia on the static hysteresis

The hysteresis studies were phased by lowering the temperature from 38°—36°C to 10°—8°C The hysteresis measurements were divided into three groups according to the temperatures 38°—30°C, 30°—20°C and

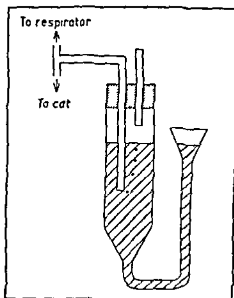


Fig 7 — The water manometer used in this work

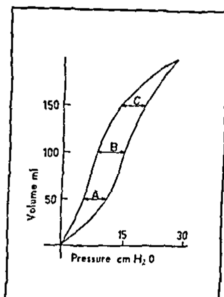


Fig 8 — The measure of hysteresis $A+B+C$

care was taken to prevent the ice from pressing on the thorax As the ice melted more was added to both ends of the basin Before the measurements were made at different temperature levels, a check was made to ensure that ice was not preventing the chest from expanding freely When the temperature reached 10°—8°C most of the ice had melted An individual experiment lasted for a maximum of three hours

In this work standard methods of statistical analysis were applied to the data obtained As has been often customary, the following expressions were used to interpret the different levels of significance

Not significant (—)	$0.05 < p$
Significant (s)	$0.01 < p \leqq 0.05$
Highly significant (h s)	$0.001 < p \leqq 0.01$
Very highly significant (v h s)	$p \leqq 0.001$

B RESULTS

1 *Static hysteresis studies*

a The measure of hysteresis

Hysteresis has generally been measured during inspiration and subsequent expiration, *i e* during the same cycle The input and output pressures have been measured at the corresponding volume levels during

inspiration and expiration The difference in the method applied in the present work was in measuring only the maximum of the pressure during the respirator stroke, the instant of zero flow The relationship between these maxima of the input and output pressures and the corresponding volumes (respirator strokes), the pressure volume curve, was measured by increasing the stroke gradually up to 200 ml and then decreasing it correspondingly The sum of the pressure differences of three volume levels (50 ml, 100 ml and 150 ml) on this curve was taken as the measure of hysteresis except the measurements performed only at a single volume level (Fig 8) This procedure ensured that the leakage of air established in the preliminary experiments especially when the stroke volume used was 100 ml or over, did not affect the results. The peak values of the tracheal pressures corresponding to the above mentioned volume levels ranged in this work

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The measuring time of hysteresis could influence the results, likewise the position of the stroke volume regulator For this reason a part of the measurements was conducted at a single volume level only The measuring time was thus as short as possible In these cases the lungs were expanded by closing the respirator expiration tube for the duration of 3—4 strokes The pressures measured at the same volume level during inspiration and expiration are thus really comparable Butler (1957) observed that the lungs and chest wall of adults upon passive deflation do not return immediately to their initial resting volume levels A return to resting values occurs after about ten seconds In static measurements the *resting expiratory* level must however be regarded as constant in fully relaxed cats breathing with a respirator and whose position is unchanged during the *experiment*

b The effect of hypothermia on the static hysteresis

The hysteresis studies were phased by lowering the temperature from 38°—36°C to 10°—8°C The hysteresis measurements were divided into three groups according to the temperatures 38°—30°C, 30°—20°C and

20°—10°C The pleural and tracheal pressure differences were measured at seven volume levels during the first ten experiments The respirator rate was 12 strokes per minute Measuring the hysteresis at seven volume levels (25 ml, 50 ml, 75 ml, 100 ml, 125 ml, 150 ml and 175 ml) by the method described took so much time that if the rate was kept at 5 strokes per minute the pulse of the subject tended to slow during the measurement As already mentioned, the sum of the pressure differences of three volume levels (50 ml, 100 ml and 150 ml) was chosen as the measure of hysteresis These sums were tabulated (Table 1) Hysteresis is expressed in cm water

The changes in the pressure volume curve of cats no 11 and 12 were measured only at these three levels in order to shorten the measuring time In these two cases a 200 ml stroke was used to expand the lungs before measuring hysteresis The results with cat no 11 (Table 1) showed that expansion of the lungs prior to measuring reduces hysteresis The mean lung hysteresis is low (0 88, 0 68 and 0 42) The same is indicated also by the results of the experiment to gauge the effect of time (Tables 2 and 3)

TABLE 1

The total respiratory hysteresis (TH) and the hysteresis of the chest wall (CH) and of the lungs (LH) in normo and hypothermia

cat no	38°—30°C			30°—20°C			20°—10°C		
	TH	CH	LH	TH	CH	LH	TH	CH	LH
1	5 8	0 8	5 0	2 5	0 0	2 5	5 6	1 5	4 1
	6 4	0 8	5 6	4 4	0 8	3 6	2 0	0 9	1 1
	3 9	1 0	2 9	2 9	0 6	2 3	0 3	0 6	—0 3
	3 6	1 0	2 6	2 7	0 5	2 2	0 4	1 1	—0 7
	2 5	0 7	1 8	1 8	0 7	1 1	2 6	0 8	1 8
	3 6	1 7	1 9	1 6	0 9	0 7	2 0	0 9	1 1
	1 7	1 7	0 0	2 0	0 8	1 2	2 7	1 7	1 0
mean	3 93	1 10	2 83	2 55	0 61	1 94	2 23	1 07	1 16
2	3 5	1 3	2 2	2 9	0 9	2 0	0 3	0 7	—0 4
	13 6	1 3	12 3	3 7	—0 6	4 3	0 4	0 1	0 3
	14 0	—1 7	15 7	2 9	1 4	1 5	0 8	0 2	0 6
	2 1	0 1	2 0	0 8	0 4	0 4	1 [illegible]	0 0	1 2
mean	8 30	0 25	8 05	2 58	0 53	2 05	0 68	0 25	0 43
3	7 1	0 6	6 5	3 9	0 2	3 7	1 0	0 0	1 0
	4 1	0 6	3 5	4 1	0 0	4 1	1 3	0 6	0 7
	5 6	0 5	5 1	0 8	0 3	0 5	1 6	0 6	1 0
	6.0	0 1	5 9	0 1	0 1	0 0	1 8	0 8	1 0
mean	5 70	0 45	5 25	2 23	0 15	2 08	1 43	0 50	0 93

4	4.5	0 2	4.3	2 0	0 6	1 4	1 4	0.3	1 1
	1 7	0 4	1.3	2 0	0 5	1.5	3 6	0 7	2 9
	2 2	0 5	1 7	1 4	0 4	1 0	1.2	—0.3	1.5
	3 5	0 6	2 9	1 7	0 2	1.5	2 0	0 2	1.8
mean	2 98	0 43	2 55	1 78	0 43	1 35	2.06	0.23	1 83
5	8 4	2 0	6 4				3 7	0 8	2 9
	6 1	1 4	4 7				2 2	0 7	1 5
	6 7	1 4	5.3				1 1	0 6	0 5
mean	7 06	1 60	5 46				2 33	0 70	1 63
6	4.9	0 1	4 8	1.3	0 3	1 0	0 9	0 1	0 8
7	4 1			4 6			2.8		
	2 7			3 4			2 2		
				1 7			2.8		
mean	3 40			3 24			2.60		
8	7 6			2 5			0 0		
	5 0			3 1			0 6		
	3 1			2 4			0 8		
mean	5 23			2 66			0 47		
9	12.9						0 9		
10	7 0						4 2		
11	1 5	0 9	0 6	2 1	1 0	1 1	1 0	0 6	0 4
	1.5	1 0	0.5	1 5	1 4	0 1	0 7	0 1	0 6
	2 5	1 0	1 5	1 0	0 4	0 6	1 3	1 0	0.3
	2 1	0 8	1.3	1 0	0 2	0 8	1 0	0 4	0 6
	1 5	1 0	0 5	0 7	—0 1	0 8	0 5	0.3	0 2
mean	1 82	0 94	0 88	1 26	0 58	0 68	0 90	0 48	0 4[illegible]

The lungs of the cats were not expanded before the measurement with the exception of the cat no 11 The sum of the pressure differences of three volume levels (50 ml, 100 ml and 150 ml) on the pressure volume curve was taken as the measure of *hysteresis* (Fig 8)

TABLE 2

Changes of the total respiratory hysteresis (TH) and the hysteresis of the chest wall (CH) and of the lungs (LH) in a course of time

cat no	0 h			1½ h			3 h		
	TH	CH	LH	TH	CH	LH	TH	CH	LH
12	2 0	0 6	1 4	3.2	0 6	2.6	3.2	0 4	2.8
	1 3	0 0	1.3	1 4	0 2	1.2	1 9	0 9	1 0
	1 3	0 6	0 7	2 7	0 6	2 1	1 4	0 4	1 0
	3 3	1 0	2 3	1.5	—1 0	2 5	1.4	1 0	0 4
	1 3	0 6	0 7	3.3	1 0	2 3	3.2	0 3	2 9
	2 1	0 6	1.5	2 8	0 4	2 4	2 3	0 8	2.3
mean	1 89	0 56	1.32	2.49	0.30	2 18	2.33	0 60	1 73

The lungs of this cat were expanded before every measurement.

20°—10°C The pleural and tracheal pressure differences were measured at seven volume levels during the first ten experiments The respirator rate was 12 strokes per minute *Measuring the hysteresis at seven volume* levels (25 ml, 50 ml, 75 ml, 100 ml, 125 ml, 150 ml and 175 ml) by the method described took so much time that if the rate was kept at 5 strokes per minute the pulse of the subject tended to slow during the measurement As already mentioned, the sum of the pressure differences of three volume levels (50 ml, 100 ml and 150 ml) was chosen as the measure of hysteresis These sums were tabulated (Table 1) Hysteresis is expressed in cm water

The changes in the pressure volume curve of cats no 11 and 12 were measured only at these three levels in order to shorten the measuring time In these two cases a 200 ml stroke was used to expand the lungs before measuring hysteresis The results with cat no 11 (Table 1) showed that expansion of the lungs prior to measuring reduces hysteresis The mean lung hysteresis is low (0 88, 0 68 and 0 42) The same is indicated also by the results of the experiment to gauge the effect of time (Tables 2 and 3)

TABLE 1

The total respiratory hysteresis (TH) and the hysteresis of the chest wall (CH) and of the lungs (LH) in normo and hypothermia

cat no	38°—30°C			30°—20°C			20°—10°C		
	TH	CH	LH	TH	CH	LH	TH	CH	LH
1	5 8	0 8	5 0	2 5	0 0	2 5	5 6	1 5	4 1
	6 4	0 8	5 6	4 4	0 8	3 6	2 0	0 9	1 1
	3 9	1 0	2 9	2 9	0 6	2 3	0 3	0 6	—0 3
	3 6	1 0	2 6	2 7	0 5	2 2	0 4	1 1	—0 7
	2 5	0 7	1 8	1 8	0 7	1 1	2 6	0 8	1 8
	3 6	1 7	1 9	1 6	0 9	0 7	2 0	0 9	1 1
	1 7	1 7	0 0	2 0	0 8	1 2	2 7	1 7	1 0
mean	3 93	1 10	2 83	2 55	0 61	1 94	2 23	1 07	1 16
2	3 5	1 3	2 2	2 9	0 9	2 0	0 3	0 7	—0 4
	13 6	1 3	12 3	3 7	—0 6	4 3	0 4	0 1	0 3
	14 0	—1 7	15 7	2 9	1 4	1 5	0 8	0 2	0 6
	2 1	0 1	2 0	0 8	0 4	0 4	1 2	0 0	1 2
mean	8 30	0 25	8 05	2 58	0 53	2 05	0 68	0 25	0 48
3	7 1	0 6	6 5	3 9	0 2	3 7	1 0	0 0	1 0
	4 1	0 6	3 5	4 1	0 0	4 1	1 3	0 6	0 7
	5 6	0 5	5 1	0 8	0 3	0 5	1 6	0 6	1 0
	6 0	0 1	5 9	0 1	0 1	0 0	1 8	0 8	1 0
mean	5 70	0 45	5 25	2 23	0 15	2 08	1 43	0 50	0 93

TABLE 5

The significance of the relative changes in the hysteresis of the chest wall (CH) and of the lungs (LH) in hypothermia

cat no	38°—30°C(I)		30°—20°C(II)		20°—10°C(III)	
	CH	LH	CH	LH	CH	LH
1	1	1	0.55	0.69	0.97	0.41
2	1	1	2.12	0.[illegible]	1.00	0.05
3	1	1	0.33	0.40	1.11	0.18
4	1	1	1.00	0.53	0.53	0.72
6	1	1	3.00	0.[illegible]1	1.00	0.17

	mean change / 10^{2}	S.E. / 10^{2}	t	P
I—II CH	+40.0	50.[illegible]	0.79	—
II—III CH	—47.8	50.5	0.94	—
I—III CH	— 7.8	10.1	0.77	—
I—II LH	—58.4	8.9	6.56	s
II—III LH	—11.0	8.5	1.29	—
I—III LH	—69.4	11.9	5.82	s

Before using t test the values of the 30°—20°C and 20°—10°C intervals (table 4) are divided by the values of the control group (38°—30°C)

c. The effect of repeated measurements on the static hysteresis in normo and hypothermia

Fig. 9 shows the effect of repeated hysteresis measurements on the pressure at a given volume level. These measurements were made at a respirator rate of 5—12 strokes per minute. The same result was achieved with numerous measurements of different animals. When the hysteresis measurements (the measure of hysteresis in this connection was the pressure difference at a given volume level) were repeated 4—5 times in succession the tracheal pressure difference at a given volume level disappeared. The changes in pleural pressure before and after repeated measurements were very small. The changes in tracheal pressure can thus be considered to derive from the lungs.

The disappearance of hysteresis was always achieved after adjusting the measuring technique to ensure that the stroke volume was really constant. The lungs were expanded between input and corresponding output pressure recording by closing the respirator expiration tube for the

TABLE 3

The significance of the variations in the hysteresis of the chest wall (CH) and of the lungs (LH) of the cat no. 12 in a course of time 0 h(I), 1½h(II) and 3 h(III)

cat no. 12		Mean	S.E.		t	p
CH	I	0.56	0.15	I—II	0.82	—
	II	0.30	0.28	II—III	0.98	—
	III	0.60	0.12	I—III	0.21	—
LH	I	1.32	0.24	I—II	2.71	s
	II	2.18	0.21	II—III	0.94	—
	III	1.73	0.43	I—III	0.83	—

Table 4 presents the variation in the mean hysteresis of the chest wall and lungs of cats no. 1, 2, 3, 4 and 6 as the temperature is lowered. The mean hysteresis of the chest wall is still practically unchanged. The mean lung hysteresis decreases from 4.70 to 1.03. For more accurate comparison between the changes, the values for 30°—20°C and 20°—10°C are divided by the control values (38°—30°C). From the relative values thus obtained the percentual variation of the changes can be seen easily. The critical ratio (t) of the chest wall shows that the chest wall hysteresis does not change significantly. In lung hysteresis, however, the change is highly significant, i.e. hypothermia appears to reduce the lung hysteresis (Table 5).

TABLE 4

The mean hysteresis of the chest wall (CH) and of the lungs (LH) in cats no. 1, 2, 3, 4 and 6

cat no.	38°—30°C		30°—20°C		20°—10°C	
	CH	LH	CH	LH	CH	LH
1	1.10	2.83	0.61	1.94	1.07	1.16
2	0.25	8.05	0.53	2.05	0.25	0.43
3	0.45	5.25	0.15	2.08	0.50	0.93
4	0.43	2.55	0.43	1.35	0.23	1.83
6	0.10	4.80	0.30	1.00	0.10	0.80
mean	0.48	4.70	0.40	1.68	0.43	1.03

TABLE 5

The significance of the relative changes in the hysteresis of the chest wall (CH) and of the lungs (LH) in hypothermia

cat no	38°—30°C(I)		30°—20°C(II)		20°—10°C(III)	
	CH	LH	CH	LH	CH	LH
1	1	1	0 55	0 69	0 9	0 41
2	1	1	2 12	0 2a	1 00	0 (5
3	1	1	0.33	0 40	1 11	0 18
4	1	1	1 00	0 53	0 53	0 72
6	1	1	3 00	0 21	1 00	0 17

	mean change / 1) ²	S E / 10^{-2}	t	P
I—II CH	+40 0	50 5	0 79	—
II—III CH	—47 8	50 5	0 94	—
I—III CH	— 7 8	10 1	0 77	—
I—II LH	—38 4	8 9	6 56	h s
II—III LH	—11 0	8 5	1 29	—
I—III LH	—69 4	11 9	5 82	h.s

Before using t test the values of the 30°—20°C and 20°—10°C intervals (table 4) are divided by the values of the control group (38°—30°C)

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Fig 9 shows the effect of repeated hysteresis measurements on the pressure at a given volume level These measurements were made at a respirator rate of 5—12 strokes per minute The same result was achieved with numerous measurements of different animals When the hysteresis measurements (the measure of hysteresis in this connection was the pressure difference at a given volume level) were repeated 4—5 times in succession, the tracheal pressure difference at a given volume level disappeared The changes in pleural pressure before and after repeated measurements were very small The changes in tracheal pressure can thus be considered to derive from the lungs

The disappearance of hysteresis was always achieved after adjusting the measuring technique to ensure that the stroke volume was really constant The lungs were expanded between input and corresponding out put pressure recording by closing the respirator expiration tube for the

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The significance of the variations in the hysteresis of the chest wall (CH) and of the lungs (LH) of the cat no 12 in a course of time 0 h(I), 1½h(II) and 3 h(III)

cat no 12		Mean	SE		t	p
CH	I	0.56	0.15	I—II	0.82	—
	II	0.30	0.28	II—III	0.98	—
	III	0.60	0.12	I—III	0.21	—
LH	I	1.32	0.24	I—II	2.71	*
	II	2.18	0.21	II—III	0.94	—
	III	1.73	0.43	I—III	0.83	—

Table 4 presents the variation in the mean hysteresis of the chest wall and lungs of cats no 1, 2, 3, 4 and 6 as the temperature is lowered. The mean hysteresis of the chest wall is still practically unchanged. The mean lung hysteresis decreases from 4.70 to 1.03. For more accurate comparison between the changes the values for 30°—20°C and 20°—10°C are divided by the control values (38°—30°C). From the relative values thus obtained the percentual variation of the changes can be seen easily. The critical ratio (t) of the chest wall shows that the chest wall hysteresis does not change significantly. In lung hysteresis, however, the change is highly significant, i.e. hypothermia appears to reduce the lung hysteresis (Table 5).

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	CH	LH	CH	LH	CH	LH
1	1.10	2.83	0.61	1.94	1.07	1.16
2	0.25	8.05	0.53	2.05	0.25	0.43
3	0.45	5.25	0.15	2.08	0.50	0.93
4	0.43	2.53	0.41	1.35	0.23	1.83
6	0.10	4.80	0.30	1.00	0.10	0.80
mean	0.48	4.70	0.40	1.68	0.43	1.03

TABLE 5

The significance of the relative changes in the hysteresis of the chest wall (CH) and of the lungs (LH) in hypothermia

cat no	38°—30°C(I) CH	38°—30°C(I) LH	30°—20°C(II) CH	30°—20°C(II) LH	20°—10°C(III) CH	20°—10°C(III) LH
1	1	1	0.55	0.69	0.9[illegible]	0.41
2	1	1	2.12	0.[illegible]	1.00	0.0[illegible]
3	1	1	0.33	0.40	1.11	0.18
4	1	1	1.00	0.53	0.53	0.72
6	1	1	3.00	0.21	1.00	0.17

	mean change / 10^3	SD / 10^3	t	p
I—II CH	+40.0	[illegible]0.5	0.79	—
II—III CH	—4[illegible].8	50.5	0.94	—
I—III CH	— 7.8	10.1	0.77	—
I—II LH	—5[illegible].4	8.9	6.56	h.s
II—III LH	—31.0	8.5	1.29	—
I—III LH	—69.4	11.9	5.92	h.s

Before using t test the values of the 30°—20°C and 20°—10°C intervals (table 4) are divided by the values of the control group (38°—30°C)

c The effect of repeated measurements on the static hysteresis in normo and hypothermia

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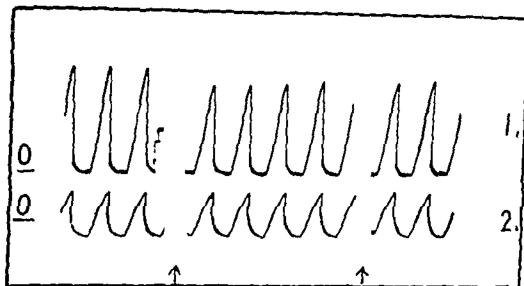


Fig 9 — The effect of repeated hysteresis measurements on the peak values of the tracheal (1) and pleural (2) pressures at the same volume level

The repeated measurements have no effect on the pleural pressure The tracheal pressure, on the contrary, decreases distinctly after the first expansion but not later The arrows show the moment of the expansion of the lungs Stroke volume 75 ml Vertical scale 1 cm = 4 cm H_2O Horizontal scale 1 cm = 6 sec

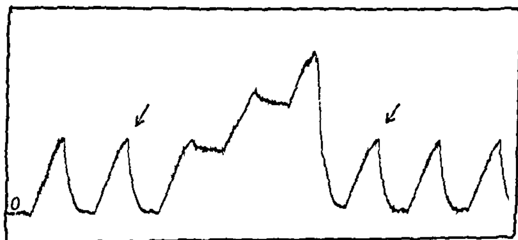


Fig 10 — The tracheal pressure is always equal before and after re expansion

The expiration tube of the respirator is closed for the duration of 3—4 strokes to allow the expansion of the lungs without changing the magnitude of the stroke to be compared The chest is open Stroke volume 75 ml Vertical scale 1 cm = 5 cm H_2O Horizontal scale 1 cm = 5 sec

duration of 3—4 strokes (Fig 10) The result was same both before and after opening the chest

The same measuring technique was employed also to establish the fact that hysteresis was zero when the lungs were expanded to any given pre-selected level and then measuring the hysteresis at any volume level lower than the pre selected level These experiments showed that no hysteresis occurred at the different temperature levels either in normothermia or hypothermia It proved possible without exception to eliminate static hysteresis by means of repeated measurements or preliminary expansion

d Static hysteresis during the same cycle Chest wall open

The lungs were expanded by 200 ml The stroke was then lowered to 75 ml The expiration tube was closed The respirator was rotated once clockwise Stabilisation of the pressure level was established with the aid of a water manometer This manometer was closed to eliminate the error caused by the movement of the water The respirator was then rotated again clockwise whereupon the corresponding quantity of air was sucked from the lungs by rotating the respirator anti-clockwise The water manometer was opened and the tracheal pressure measured The pressure values obtained before and after the clockwise and anti clockwise movement of the respirator were compared If the movement was slow nearly static the starting pressure was higher than the pressure measured after the anti-clockwise movement The pressure difference ranged from 0 5 to 4—5 cm water By contrast if the anti clockwise and clockwise movement was performed in 1—2 seconds the pressure differences disappeared and the pressure was equally great before and after the dynamic respirator movement (Fig 11)

2 Air leakage from the lungs

a. Excised lungs

Cat lungs removed as atraumatically as possible were sealed hermetically in the tracheal cannula the side arm of the T tube The T tube was connected with the respirator and the water manometer The air tightness of the joints was controlled by the water manometer by closing

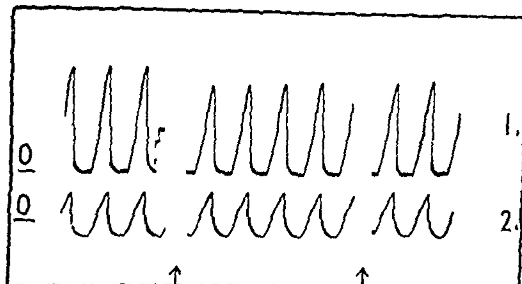


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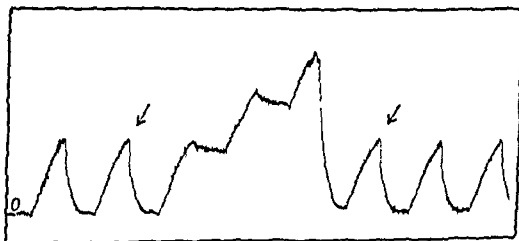


Fig 10 — The tracheal pressure is always equal before and after re expansion

The expiration tube of the respirator is closed for the duration of 3—4 strokes to allow the expansion of the lungs without changing the magnitude of the stroke to be compared The chest is open Stroke volume 75 ml Vertical scale 1 cm = 5 cm H_2O Horizontal scale 1 cm — [illegible] sec



Fig 12 — The tracheal pressure caused either by increasing the quantity of air in the lungs gradually (8 × 25 ml) or by a single 200 ml stroke

The chest is open Vertical scale 1 cm = 10 cm H_2O Horizontal scale 1 cm = 10 sec

was then rotated once clockwise and back again The water manometer was opened and the pressure in the airways noted Compared with the measurements mentioned in item 1d these showed that the pressure in the airways equalled the starting pressure or differed from it by no more than 0 5 cm water also after the considerably slower, near static clockwise and anti clockwise movement of the respirator

25 ml was chosen as the respirator stroke The expiration tube was *closed* The respirator rate was 5 *strokes per minute* Fig 12 shows the changes in the tracheal pressure with the *chest open* The respirator expiration tube was opened after the 8th stroke The same procedure was repeated several times The result was repeatedly as *shown in* Fig 12 The pressure fell hardly at all after the first stroke (25 ml), but *the greater* the quantity of air in the lungs as a result of *repeated* strokes, the sharper was the pressure drop after the stroke Using a *graduated* glass, 8 successive strokes and the quantity of air leaving the lungs *after* the 8th stroke were both measured The former was 200 ml ± 5 ml, the latter 160 ml ± 5 ml A comparable pressure drop was observed irrespective of how slowly the measurement was performed The same result was achieved in an investigation performed on a dead animal

The respirator stroke was raised to 200 ml and the resulting pressure increase was measured Fig 12 *shows that* the pressure caused by a gradually rising stroke (8×25 ml) was clearly lower *than the pressure caused* by a single one (200 ml) When repeated measurements were made, the difference was found to be 20—25 per cent, in other words, the *former*

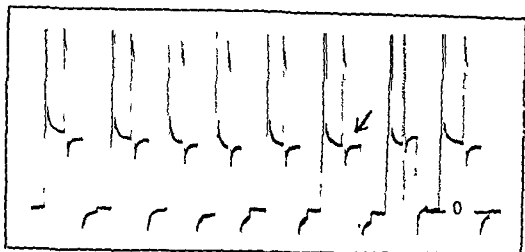


Fig 11 — The tracheal pressure is of the order of the same magnitude before and after the clockwise and anti clockwise movement of the respirator
If this movement of the respirator is made in 1—2 seconds the pressure difference disappears The chest is open Stroke volume 75 ml Vertical scale 1 cm = 4 cm H_2O

different parts of the tubes in turn In spite of this, the pressure caused by the respirator stroke was reduced immediately after the stroke although the expiration tube was closed unless the stroke volume was less than 50 ml The procedure described above was repeated after immersing the lungs *in the water by pressing them under with the hand The formation* of small air bubbles from the surface of the lungs was noted It increased with the stroke volume

b Intact lungs Chest wall open

The above experimental series was repeated without excising the lungs from the animal The chest wall was opened sternally When the chest cage had been filled with saline solution the same bubbling was noted but *considerably less profusely than in the excised lungs*

The chest cage was filled with paraffin oil instead of saline solution The experiment described in section 1d was repeated 75 ml was selected as the respirator stroke *The lungs were expanded with the expiration* tube closed When the movement of the water manometer had ceased and the pressure was stabilized the water manometer was closed The respirator



Fig 12 — The tracheal pressure caused either by increasing the quantity of air in the lungs gradually (8 × 25 ml) or by a single 200 ml stroke
The chest is open. Vertical scal 1 cm = 10 cm H_2O Horizontal scale 1 cm = 10 sec

was then rotated once clockwise and back again The water manometer was opened and the pressure in the airways noted Compared with the measurements mentioned in item 1d, these showed that the pressure in the airways equalled the starting pressure or differed from it by no more than 0 5 cm water also after the considerably slower, near static clockwise and anti clockwise movement of the respirator

25 ml was chosen as the respirator stroke The expiration tube was *closed* The respirator rate was 5 strokes per minute Fig 12 shows the *changes in the tracheal pressure* with the chest open The respirator *expiration* tube was opened after the 8th stroke The same *procedure was* repeated several times. The result was repeatedly *as shown in Fig 12* The pressure fell hardly at all after the first stroke (25 ml), but the greater the quantity of air in the lungs as a result of repeated strokes, the sharper was the pressure drop after the stroke Using a graduated glass, 8 successive strokes and the quantity of air leaving the lungs after the 8th stroke were both measured The former was 200 ml ± 5 ml, the latter 160 ml ± 5 ml A comparable pressure drop was observed irrespective of how slowly the measurement was *performed* The same result was achieved in an investigation performed on a dead animal

The respirator stroke was raised to 200 ml and the resulting pressure increase was measured Fig 12 shows that the pressure caused by a gradually rising stroke (8×25 ml) was clearly lower than the pressure caused by a single one (200 ml) When repeated measurements were made, the difference was found to be 20—25 *per cent*, in other words, the former

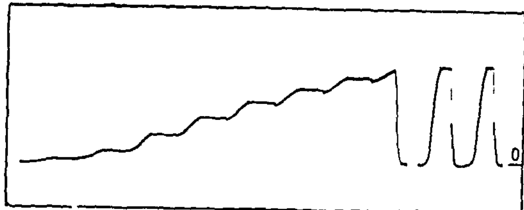


Fig 13 — The tracheal pressure caused either by increasing the quantity of air in the rubber bag gradually (8×25 ml) or by a single 200 ml stroke
The chest is open Vertical scale 1 cm = 10 cm H_2O Horizontal scale 1 cm = 10 sec

was roughly ¼ of the latter In order to eliminate possible leakage in the measuring apparatuses, the T tube was removed from the lungs and an elastic rubber bag was substituted for the lungs *Fig* 13 presents the results It shows the stability of the pressure level after every gradual rise and the equal size of the maximum pressures caused by a single stroke (200 ml) or achieved by gradually increasing volume in the lungs

3 *Static elastance studies*

a Method

The lungs were expanded by 200 ml at ten minute intervals and just before making the measurement Expansion was achieved by closing the expiration tube of the respirator for the duration of 3—4 strokes, depending on the magnitude of the stroke The stroke thus remained unchanged throughout the investigation Static elastance was measured in normothermia and at temperatures gradually lowered to 30°—28°C 20°—18°C and 10°—8°C As the animal is completely relaxed, the pleural pressure changes illustrate the role of the chest wall and its variations It is possible to measure the total respiratory elastance and its variations by recording the tracheal pressure and noting the respirator stroke volume On the other hand, the sum of *the elastance values of the chest wall and the lungs*

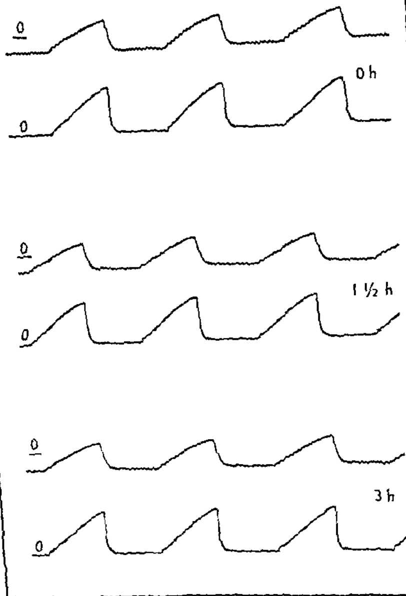


Fig 14 — The effect of time on the tracheal (lower) and pleural (upper) pressures
The lungs were expanded before the measurement Stroke volume 50 ml Vertical scale 1 cm = 4 5 cm H_2O Horizontal scale 1 cm = 4 sec.

is equal to the total elastance The simple comparison of the tracheal and pleural pressure fluctuations gives thus a picture of the changes in the total respiratory elastance, the chest wall and the lung elastance in normo and hypothermia, since the respirator stroke is constant

Since successive pleural and tracheal pressure values do not differ much mutually, 0 2 cm water at the most, only the mean pressures of each subject for the different temperatures have been tabulated Fig 14 *shows the similarity of these successive measurements It was felt that* more detailed statistical analyses were unnecessary in this connection, and so the value for each control group (38°—36°C) was denoted by 1 and, for *comparison of the mean pressures, the values for the different temperature* levels were divided by the control value (Tables 6 and 7) As the stroke volume was constant for each animal, these relative values illustrate at the same time the relative variations in chest wall and lung elastance at different temperature levels The same procedure was adopted in studying the time factor (Tables 8 and 9) The critical ratio was only used for analysis of these relative values and the significance of their differences

TABLE 6

Changes of the tracheal (Ptr), pleural (Ppl) and transpulmonary (Ptp) pressures in normo and hypothermia (cm H O)

cat no	38°—36°C Ptr	Ppl	Ptp	30°—28°C Ptr	Ppl	Ptp
26	5 9	3 4	2 5	6 2	3 6	2 6
27	5 3	3 6	1 7	5 9	4 2	1 7
28	6 2	3 9	2 3	7 3	4 8	2 5
29	7 8	3 6	4 2	8 7	4 5	4 2
30	5 4	2 5	2 9	5 9	3 1	2 8
31	6 2	3 6	2 6	6 2	3 6	2 6
32	7 6	3 9	3 7	7 6	4 2	3 4

cat no	20°—18°C Ptr	Ppl	Ptp	10°—8°C Ptr	Ppl	Ptp
26	6 2	3 6	2 6	7 2	4 3	2 9
27	5 9	4 2	1 7	9 0	5 3	3 7
28	7 8	5 0	2 8	10 3	6 2	4 1
29	8 5	4 5	4 0	9 2	4 8	4 4
30	6 7	3 9	2 8	8 0	4 9	3 1
31	6 0	3 5	2 5	7 0	4 3	2 7
32	7 8	4 2	3 6	9 2	5 3	3 9

The corresponding stroke volumes ranged from 30 ml to 75 ml

TABLE 7

The significance of the relative changes in the chest wall (Ce) and lung (Le) elastance in hypothermia

cat no	38°—36°C(I)		30°—28°C(II)		20°—18°C(III)		10°—8°C(IV)	
	Ce	Le	Ce	Le	Ce	Le	Ce	Le
26	1	1	1 06	1 04	1 06	1 04	1 27	1 16
27	1	1	1 17	1 00	1 17	1 00	1 47	2 19
28	1	1	1 23	1 09	1.28	1 22	1,59	1 79
29	1	1	1.25	1 00	1.25	0 95	1 33	1 05
30	1	1	1 24	0 97	1 56	0 97	1 96	1 07
31	1	1	1 00	1 00	0 97	0 96	1 20	1 04
32	1	1	1 08	0 92	1 08	0 97	1,36	1 06

	mean change / 10^{2}	S E / 10^{2}	t	p
I—II Ce	+14 7	3 78	3 88	h s
II—III Ce	+ 4 9	4 56	1 07	—
III—IV Ce	+25 8	3 78	6 82	v h s
I—II Le	+ 0 3	2 00	0 15	—
II—III Le	+ 1 3	2 30	0 57	—
III—IV Le	a +32 0	15 8	2 02	—
	b + 9 6	0 66	14.8	v h s

Before using t test the pressure values of the 30°—28°C, 20°—18°C and 10°—8°C levels (table 6) are divided by the values of the control group (38°—36°C) The stroke volume was constant during every experiment Thus the above values correspond also to relative changes of the elastance

b The effect of hypothermia on the static elastance

— The chest is closed

It can be seen from Tables 6 and 7 that the elastance of the chest wall increased by an average of 14 7 per cent when the temperature was lowered from 38°—36°C to 30°—28°C This change of the chest wall elastance is highly significant When the temperature was lowered from 30°—28°C to 20°—18°C however the average change was no more than 4 9 × 10 ² The elastance of the chest wall remained unchanged during this time in as many as 4 cats Chest wall elastance values measured in deep hypothermia, 10°—8°C were without exception higher than the values at 20°—18°C, the mean difference was 25 8 × 10 ², when the value of the control group

is equal to the total elastance The simple comparison of the tracheal and pleural pressure fluctuations gives thus a picture of the changes in the total respiratory elastance, the chest wall and the lung elastance in normo and hypothermia, since the respirator stroke is constant

Since successive pleural and tracheal pressure values do not differ much mutually, 0 2 cm water at the most, only the mean pressures of each subject for the different temperatures have been tabulated Fig 14 shows the similarity of these successive measurements It was felt that more detailed statistical analyses were unnecessary in this connection, and so the value for each control group (38°—36°C) was denoted by 1 and, for comparison of the mean pressures, the values for the different temperature levels were divided by the control value (Tables 6 and 7) As the stroke volume was constant for each animal, these relative values illustrate at the same time the relative variations in chest wall and lung elastance at different temperature levels The same procedure was adopted in studying the time factor (Tables 8 and 9) The critical ratio was only used for analysis of these relative values and the significance of their differences

TABLE 6

Changes of the tracheal (Ptr), pleural (Ppl) and transpulmonary (Ptp) pressures in normo and hypothermia (cm H O)

cat no	38°—36°C			30°—28°C		
	Ptr	Ppl	Ptp	Ptr	Ppl	Ptp
26	5 9	3 4	2 5	6 2	3 6	2 6
27	5 3	3 6	1 7	5 9	4 2	1 7
28	6 2	3 9	2 3	7 3	4 8	2 5
29	7 8	3 6	4 2	8 7	4 5	4 2
30	5 4	2 5	2 9	5 9	3 1	2 8
31	6 2	3 6	2 6	6 2	3 6	2 6
32	7 6	3 9	3 7	7 6	4 2	3 4

cat no	20°—18°C			10 —8 C		
	Ptr	Ppl	Ptp	Ptr	Ppl	Ptp
26	6 2	3 6	2 6	7 2	4 3	2 9
27	5 9	4 2	1 7	9 0	5 3	3 7
28	7 8	5 0	2 8	10 3	6 2	4 1
29	8 5	4 5	4 0	9 2	4 8	4 4
30	6 7	3 9	2 8	8 0	4 9	3 1
31	6 0	3 5	2 5	7 0	4 3	2 7
32	7 8	4 2	3 6	9 2	5 3	3 9

The corresponding stroke volumes ranged from 50 ml to 75 ml

is 1. This change can be regarded as very highly significant ($p < 0.001$). It can be stated that deep hypothermia indeed increases the elastance of the chest wall (Table 7 and Fig 15).

It can be seen from Tables 8 and 9 that the lung elastance in the course of time changes in one direction or another by 10—14 per cent at the most (Fig 14). The corresponding values for 30°—28°C and 20°—18°C are well within these limits. The only exception is the cat no 28. At 10°—8°C, on the other hand, all the values were higher than the control value. Four of them rose by 4—8 per cent, one by 16 per cent and two exceptionally much by 79 and 118 per cent, respectively. Dorsally located atelectatic areas, considerably larger than in any other lungs, were demonstrated at autopsy in the lungs of these last two subjects. Furthermore the airways of these two animals had to be cleared 2—3 times during the experiment. Extensive opening of the airways at autopsy showed, however, that all the lungs were free of mucus after the experiment. The critical ratios of the lung elastance changes were recalculated excluding these two animals. Disregarding these exceptionally high values it can in fact be asserted that lung elastance changes were very highly significant when the temperature is lowered from 20°—18°C to 10°—8°C.

TABLE 8

Changes of the tracheal (Ptr), pleural (Ppl) and transpulmonary (Ptp) pressures in a course of time (cm H_2O)

cat no	0 h			1½ h			3 h		
	Ptr	Ppl	Ptp	Ptr	Ppl	Ptp	Ptr	Ppl	Ptp
33	5.6	3.9	1.7	5.3	3.8	1.5	5.6	3.8	1.8
34	5.0	2.8	2.2	5.6	3.1	2.5	5.6	3.1	2.5
35	5.3	3.9	1.4	5.5	3.9	1.6	5.2	3.6	1.6
36	6.3	3.2	3.1	6.5	3.4	3.1	6.4	3.4	3.0

The lungs of the cats were expanded before every measurement

TABLE 9

Relative changes in the chest wall (Ce) and lung (Le) elastance in a course of time

cat no	0 h		1½ h		3 h	
	Ce	Le	Ce	Le	Ce	Le
33	1	1	0.98	0.89	0.98	1.06
34	1	1	1.10	1.14	1.10	1.14
35	1	1	1.00	1.14	0.92	1.14
36	1	1	1.06	1.00	1.06	0.97

The pressure values of the 1½ h and 3 h (table 8) are divided by the values of the control group (0 h). The stroke volume was constant during every experiment. Thus the above values correspond also to relative changes of elastance.

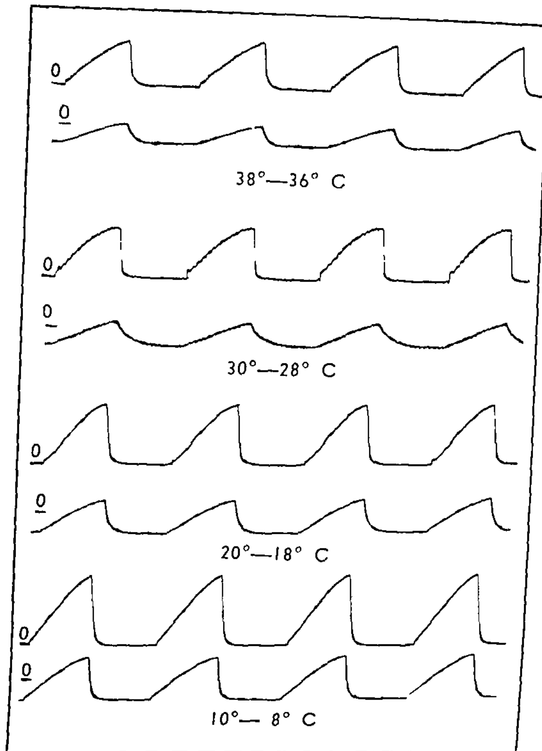


Fig 15 — The effect of hypothermia on the tracheal (upper) and pleural (lower) pressures

The lungs were expanded before the measurement. Stroke volume 50 ml. Vertical scale 1 cm = 4.5 cm H_2O. Horizontal scale 1 cm = 4 sec

ml To establish the restricting effect of the ice on the expansion of the chest, wood pulp (weighing 200 g) moistened with water was placed on the chest of the subject The mean pressures before and after adding the weight were 5 6 cm water and 6 4 cm water Fig 17 shows the fluctuations in pleural pressure during the emptying of water from the basin Fig 16 illustrates the variations in pleural pressure before and after the weight was added and when it was removed again These figures reveal the small effect of the addition of water, whereas the 200 g weight causes a spontaneous and immediately stabilizing pressure increase

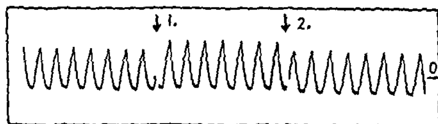


Fig 16 — The pleural pressure before and after adding a 200 g weight on the chest of a cat

1 The weight is added 2 The weight is removed Stroke volume 100 ml Vertical scale 1 cm = 5 cm H_2O

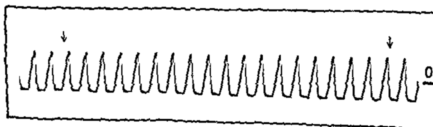


Fig 17 — The pleural pressure when the basin is being emptied

Stroke volume 100 ml Vertical scale 1 cm = 5 cm H_2O

— The water manometer experiments The chest is open

The lung was expanded by 200 ml The water manometer was used as the water valve This water valve was set to correspond to the maximum of the pressure caused in the airways by the stroke The water valve was closed Re expansion by 200 ml was performed The water valve was opened *in order to control that its limit value really corresponded to the maximum* of the pressure It was noted at ten minute intervals, by expanding the lungs by 200 ml and opening the water valve that the maximum pressure remained unchanged during the lowering of the temperature It the bubbling from the water valve was noted as evidence of an increase in maximum *pressure, expansion was repeated and the maximum pressure controlled* Table 10 shows the results with three cats The maximum of the pressure level remained unchanged down to 20°—18°C in all the cats *i e* it was possible to return the maximum of the pressure, which may have risen momentarily to some extent, to the control level (38°—36°C) by re expanding the lungs by 200 ml *On the other hand*, when the temperature was lowered to below 20°—18°C, and especially when cardiac function slowed down and the temperature approached 10°—8°C, this return began to meet with difficulties The bubbling from the water valve continued despite repeated expansion The control carried out at 10°—8°C by raising the limit value of the water valve showed that the maximum pressure had risen 0 4—0 6 cm water above the control values in all three cases *As the table shows* the pressures at 50—60 ml volume level were 4 5—5 8 cm water

TABLE 10

Changes in the lung elastance measured by means of the water manometer in normo and hypothermia (cmH_2O/ml)

cat no	38°—36°C	30°—28°C	20°—18°C	10°—8°C
37	4 5/50	4 5/50	4 5/50	4 9/50
38	5 2/60	5 2/60	5 2/60	5 8/60
39	4 8/60	4 8/60	4 8/60	5 3/60

c The restricting effect of water and ice on chest expansion

The average values of 50 pleural pressure measurements before and after the *addition of water into the basin were almost equivalent* 5 7 cm water and 5 6 cm water *Fig* 17 shows that the deviation was practically

ml To establish the restricting effect of the ice on the expansion of the chest wood pulp (weighing 200 g) moistened with water was placed on the chest of the subject The mean pressures before and after adding the weight were 5 6 cm water and 6 4 cm water Fig 17 shows the fluctuations in pleural pressure during the emptying of water from the basin Fig 16 illustrates the variations in pleural pressure before and after the weight was added and when it was removed again These figures reveal the small effect of the addition of water, whereas the 200 g weight causes a spontaneous and immediately stabilizing pressure increase

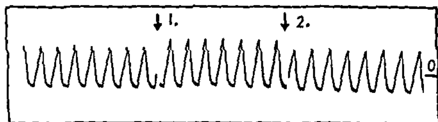


Fig 16 — The pleural pressure before and after adding a 200 g weight on the chest of a cat

1 The weight is added 2 The weight is removed Stroke volume 100 ml Vertical scale 1 cm = 5 cm H_2O

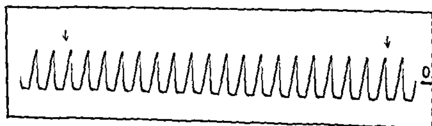


Fig 17 — The pleural pressure when the basin is being emptied

Stroke volume 100 ml Vertical scale 1 cm = 5 cm H_2O

V. DISCUSSION

Changes in posture were found by Safar and Aguto Escarraga (1959) to cause variations in compliance. Many other workers have come to the same conclusion *(Attinger, Monroe and Segal 1956, Lim and Luft 1959,* Ferris, Mead and Frank 1959). Satisfactory results in static respiratory mechanics experiments were achieved by Buytendijk (1949), Butler, White and Arnott (1957) and Ehrner (1960) by making the subject breathe as slowly as possible. Butler (1957) observed that the lungs and chest wall of adults upon passive deflation do not return immediately to their initial resting volume levels after inflation to 20 cm water pressure. *A return to resting values occurs after about ten seconds. Recording of* esophageal pressure has been used as a substitute for pleural pressure measurements (Buytendijk 1949, Dornhorst and Leathart 1952). The concurrent measurement of these pressures have now shown, however, that they differ from one another (Butler, White and Arnott 1957, Mead and Gaensler 1959). Furthermore, it has been found by Coleridge and Linden (1954) and Farhi, Otis and Proctor (1957) that the pleural *pressure at various points over the surface of lungs is not identical.*

The total respiratory compliance values measured in conscious persons with the aid of the relaxation method have been found to be too high (Nims, Conner and Comroe 1955). In this connection the author points to the figure 4 in the present work. The decrease of the compliance measured during anesthesia was noted *e.g.* by Holaday and Israel (1955), Wu, Miller and Luhn (1956) and Howell and Peckett (1957). Safar and Bachman *(1956) studied apneic dogs anesthetized with pentobarbital found how*ever, that the total respiratory compliance remained unchanged although the depth of anesthesia varied provided that I.P.P.B. was *constant.* D-tubocurarine *lowers the total respiratory compliance in dogs (Safar and Bachman 1956, Massion 1957).* Safar and Bachman established, *on* the contrary, that suxamethonium does not cause changes in compliance in dogs. It was observed by Mead and Collier (1959) that the lung compliance is at its maximum immediately after forced inflation. This compliance in a course of time decreased slowly, but could always be

returned through more vigorous inflation to near maximal level Hypothermia led to apnea.

For these reasons, the present author used cats breathing with the aid of respirator, anesthetized with pentobarbital and relaxed with suxamethonium The slowest respirator rate was 5 strokes per minute The cats were kept in the same supine position throughout the experiment The pleural pressure measurement was employed The pleural catheter was at the same point during each experiment The lungs were expanded *before the measurement in order to eliminate* the effect of time The values compared for *normothermia and* hypothermia measurements were always for the *same animal* Cats were used because *they* are inexpensive and very stable *even during* a long experiment

Stress relaxation has been demonstrated by several investigators (Bayliss and Robertson 1939 Mount 1955 Butler 1957), but few have *tried to account for it An extensive study* of the phenomenon was made by Hughes May and *Widdicombe (1959) Ruling* out all other possible causes, they arrived at *the* assumption that stress relaxation is due to a yielding of pulmonary tissue in addition to surface tension effects, and it is comparable with the stress relaxation seen in other tissues This was confirmed by Marshall and Widdicombe (1961) They did not consider leakage of air from the lungs as a possible reason

Katz (1909) and Kronecker (1909) noted that air may *begin to leak* through the *connective tissue of* the bronchioles and this produces interstitial emphysema *in* the neck or abdomen at higher pressures of 40 to 50 mmHg *Henry (1945)* stated that at still higher pressures of 60 to 100 mmHg the lungs are likely to rupture From the present investigations it seems probable that this air leakage occurs already at a considerably lower pressure

As can be seen from Fig 12 25 ml does not induce the stress relaxation The next stroke increases the amount of air in the lungs and a slight pressure drop then occurs after the stroke This *fall* in tracheal pressure increases stroke by stroke It is already *considerable* after the 7th stroke The air tightness of the measuring apparatuses was controlled by using the rubber bag as a substitute for the lungs (*Fig* 13) The pressure drop must therefore be regarded as deriving *from the* lungs because the chest was open On the other hand experiments performed on excised lungs suggest strongly that the leakage of air is the cause of the pressure drop The cat lungs were removed as carefully as possible This notwithstanding there was profuse formation of small bubbles *when the* lungs were

pressed under the surface of water The same phenomenon occurred when the chest cage was filled with saline solution, although considerably more weakly

The respirator *operation was interrupted while the chest was being* cautiously opened from the xiphoid angle The lungs now collapsed The damage to the lungs during the chest opening does not account for the leakage of air When 8 successive respirator strokes were measured pneumatically, the sum of these was 200 ml ± 5 ml When the quantity of air leaving the lungs *after the 8th stroke was measured, it was only 160* ml ± 5 ml A difference of corresponding magnitude was also established between the peak value of the pressure after the 8th stroke and the pressure caused by a single stroke of 200 ml (Fig 12) No corresponding difference was observed when the rubber bag was used as a substitute for the lungs (Fig 13)

The change occurring both in the quantity of air and the pressure is about 20 per cent The shifting of the end expiratory level seems improbable as a factor affecting the result, for even the results of as many as 10 repeated measurements show a difference of no more than ± 5 ml The significance of air leakage is confirmed by *the observation that* when the chest was open it was possible to eliminate the pressure difference measured before and after the clockwise and anti clockwise movement of the respirator only by very dynamic action (Fig 11) In dynamic action there is not enough time for air to be discharged from the lungs By filling the chest cage with paraffin oil which inhibits air leakage at least to some extent it was possible also when acting considerably more slowly to establish that the pressures *had become equal before and after* the respirator clockwise and anti clockwise movements

It is possible that the method used in the present work has an effect on the results But the differences are so great (200 ml — 160 ml) that air leakage can be stated to be a factor causing static hysteresis and stress relaxation *If this possibility is not taken into consideration* in the choice of the method a notable misinterpretation of the results can ensue In the author s opinion no earlier investigation *contains an argument* against air leakage

Several observations suggest that the lungs are lined by a fluid layer with intense surface activity (*e g* Terry 1926 Macklin 1954 Brown Johnson and Clements 1959) Mead Whittenberger and Radford (1957) and Radford (1957) emphasized particularly the contribution of surface tension as the cause of static hysteresis They held that this was substantiated by

the fact that the hysteresis in connection with air filling disappears almost completely when the pressure volume curve is measured by filling the lungs with saline solution *Radford* (1957) drew attention to the biphasic character of the pressure volume curve due to the opening pressure phenomenon during inspiration This opening pressure effect has been demonstrated as early as 1934 by Lindskog and Bradshaw, and 1947 by Gruenwald

Differing from the above Agostoni *et al* (1958) established marked hysteresis during liquid filling of the lungs of mature guinea pig, cat and goat fetuses On the basis of this observation they reasoned that the similarly marked hysteresis of gas filled adult lungs is due to tissue *properties* They claimed that this view is also supported by *the fact, already* shown in the adult lung (Setnikar *1955*, Agostoni and *Taglietti 1957*) *that* the slower the rate of inflation and deflation the larger the *hysteresis* area According to the present work the last mentioned event is at least *due* partly to the leakage of air from the lungs

On the other hand Radford and Lefcoe (1955) found only small changes in retractive pressures following induced contraction and relaxation of the smooth muscle in the tracheobronchial tree This conclusion is supported by Mead and Collier *(1959)* and Pierce Hocott and Hefley (1961) They *claimed that* the bronchial smooth muscle and the amount of blood in the pulmonary vascular bed *are factors of* minor importance in the static *hysteresis although* it has been shown by Borst *et al* (1957) that in dogs acute changes in *pulmonary arterial* and venous pressures are associated with changes in the mechanical *behaviour* of the lungs

Clements (1957) was *the* first to point out that a low coefficient of surface film compressibility *(which expresses* the sharp drop off tension during deflation) would have a *stabilizing influence* if present in the air spaces Brown Johnson and *Clements (1959)* stated that surface tension diminishes considerably *during expiration* They investigated the area surface tension hysteresis of surface *film* prepared from cat s lung extract The process is reversible if this *film is compressed* to about 50 per cent of its surface area but with further compression the film appears to rupture on re expansion Clements *et al* (1961) noted that in normal rat lungs, the area surface tension hysteresis was considerable whereas it disappeared in rat lungs treated with a nonionic detergent *In the* last mentioned case the lungs had the greatest tendency to become atelectatic The static surface tension on the other hand is the equilibrium tension the dynamic tension is the tension of a liquid before the surface film has had time to form *(Bull*

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1951) With solutions of colloidal materials the time required for the formation of the surface is appreciable, too It seems that Brown *et al* in fact measured the dynamic and not the true, static surface tension of the above mentioned film Hence viscosity of the liquid had a notable *role in* the achievement of the results obtained, and the viscosity of colloidal materials is known to be great The rupturing of the surface film on re expansion is probably due to the considerably smaller number *of liquid molecules in this film*, since a *part of the liquid forming the* surface film has become sedimentated in the course of time under the influence of gravity It seems that the dynamic tension, the opening and *closing pressure of alveoli and the viscosity of the liquid play an important* part in these changes

Three air spaces stabilizing factors were quoted by Mead (1961) geometric *stability, surface film stability and the stabilizing effect of the* tissue surrounding the alveoli The first is the case that Neergaard (1929) considered where all surfaces are less than hemispheres and radii of curvature decrease as volume increases The second factor operates beyond the hemispheric shape and involves the change in surface tension with area discovered by Brown and by Clements

In the present author s opinion alveolar stability has a central role in the static hysteresis phenomenon As long as this stability is preserved no static hysteresis occurs He observed the pressure changes at a given volume level when the stroke volume was increased and decreased To eliminate the error caused by air leakage, the experiment was conducted stroke by stroke starting from the resting expiratory level The rate of the respirator was 5 strokes per minute So there were more than 10 seconds between each stroke The end expiratory level thus remained unchanged The peak values of the input and output pressures caused by the same quantity of air were in fact compared The lungs were expanded between the input and corresponding output pressure recording by *closing* the expiration tube of the respirator for the duration of a few strokes The position of the stroke volume regulator was thus constant *during* the measurements

It was found that static hysteresis always disappeared on taking repeated measurements (Figs 9 and 10) When the lungs were expanded to any *pre-selected level* and hysteresis was then measured at any volume level below the selected level, the hysteresis was still nil It may be mentioned that Bernstein (1957) observed the similarity of the 2nd and subsequent measurements of the pressure-volume curve

It was shown by Donders (1849) that the opening of the chest causes the collapse of the lungs. The chest expands on the other hand because the neutral position of the chest wall is at 70 per cent vital capacity level. It was established by Mead and Collier (1959) that compliance was at its maximum immediately after forced inflation and at its minimum following forced deflation. After forced inflation compliance in a course of time was reduced rapidly whereas it remained unchanged after forced deflation. Pierce, Hocott and Hefley (1961) noted that the ratio of surface area to volume diminishes with the volume. They concluded this must have resulted from the closure of some of the smaller air spaces during expiration. This substantiates the present author's conception of alveolar stability. When it is disturbed, *e.g.* on expanding the lungs, a part of the alveoli tend to close immediately. Only the number of alveoli corresponding to the respirator stroke volume used remain open. In consideration of the above the thorax, the respirator stroke volume, non-elastic resistance of the chest wall, the lungs and the respirator during expiration and surface tension as a force preventing the opening of the alveoli in particular or closing them constitute together an entity. This entity tends effectively to moderate sudden changes in the lungs and to stabilize the lungs as soon as possible. It is probable that the viscosity of alveolar fluid plays an important part in the opening pressure phenomenon discussed by *Agostoni et al.* (1958). It is possible that this viscosity is one of the factors which tends to hold the alveoli open during expiration.

Static hysteresis in hypothermia has probably not been studied earlier. The first stage of the present studies led to the observat[illegible]

[illegible] significant change in lung hysteresis between the 0 h and 1½ h values (Tables 2 and 3). Although only a few measurements were made to study the effect of the time factor, the appreciably greater similarity of these measurements in normothermia compared with the results of the control group (38°—36°C) attracted the attention. In analysing the reasons for this the author found that he had acted differently in one respect. The lungs were expanded by 200 ml in the time factor experiment before measuring the hysteresis. A notable reason for the different results was probably the absence of preliminary expansion in a considerable number of the measurements. On the other hand, when the temperature was lowered the lungs expanded again and again in the repeated measurements. As mentioned earlier these in

1951) With solutions of colloidal materials the time required for the formation of the surface is appreciable too It seems that Brown *et al* in fact measured the dynamic and not the true static surface tension of the above mentioned film Hence viscosity of the liquid had a notable role in the achievement of the results obtained and the viscosity of colloidal materials is known to be great The rupturing of the surface film on re-expansion is probably due to the considerably smaller number of liquid molecules in this film since a part of the liquid forming the surface film has become sedimentated in the course of time under the influence of gravity It seems that the dynamic tension the opening and closing pressure of alveoli and the viscosity of the liquid play an important part in these changes

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It was found that static hysteresis always disappeared on taking repeated measurements (Figs 9 and 10) When the lungs were expanded to any pre-selected level and hysteresis was then measured at any volume level below the selected level the hysteresis was still nil It may be mentioned that Bernstein (1957) observed the similarity of the 2nd and subsequent measurements of the pressure-volume curve

a noticeable rise in the elastance of the chest wall and lungs. It can be claimed that the rise was very highly significant ($p \leqq 0.001$). The mean increase in the elastance of the chest wall is 25.8 per cent. The corresponding value in lung elastance is 9.8 per cent if cats no 27 and 28 are excluded. The corresponding critical ratios are 6.82 and 14.8. Lung elastance experiments with the water manometer confirm this increase (Table 10).

What are these changes due to? Three observations are of importance in arriving at an explanation. One of them concerns cardiac function. When the temperature was lowered from 20°—18°C to 10°—8°C, the pulse slowed abruptly. Electrical activity was no longer recorded in ECG at 12°—13°C. Prior to this it was possible to detect a few deflections repeated at irregular intervals. The more pronounced changing of the lung elastance of cats no 27 and 28 occurred after this when the temperature was lowered to the 10°—8°C level. It should be noted that the rate of temperature fall had steadily slowed and that the changes in lung elastance did not take place immediately after electrical activity ceased to be recorded in ECG *but in the course of about 30 minutes*. The second observation concerns the sudden changes at the 20°—15°C level. It was found on 3—4 occasions when the expansion of the lungs at ten minute intervals was neglected for one reason or another that lung elastance had risen sharply. Despite subsequent repeated expansion lung elastance tended to remain elevated *until* a sudden change occurred again and the elastance fell to the control level. The third observation concerns the autopsy after the experiment. On autopsy Drinker and Hardenbergh (1948) noted the congested and liverlike appearance of lungs of dogs kept under deep anesthesia and *held supine* for some hours. Mead and Collier (1959) presented a series of pictures showing that these dark areas of lungs established on autopsy can be *eliminated by* inflation. In the present work on the contrary, although the lungs were *expanded* in the same way as during the experiment, small atelectatic areas which were never encountered at higher temperatures *in similar* conditions occurred in the dorsal parts of the lungs (in the lungs of cats no 27 and 28 there were exceptionally large atelectatic areas).

Greater hysteresis is found after death (Hirakawa 1924 and Dean and Visscher 1941) and *in* the exsanguinated lung (Radford, Lefcoe and Mead 1954) than in the lung with intact circulation. Avery, Frank and Gribetz (1959) presented a *pressure* volume curve which showed that collapsed lungs are easier to inflate when the vascular bed of the lungs has been filled with saline solution. Peltonen and Kreiner (1961) stated that the

vestigations were carried out at a respirator rate of 12 strokes per minute. The results were thus possibly affected also by dynamic factors. Furthermore, it is probable that insufficient attention was paid to the relaxation of the subject.

After checking that the successive measurements really did lead to the reduction of hysteresis, the comparative hysteresis studies were continued at one and the same volume level. The decisively shortened measuring time of the hysteresis reduced considerably the effects of measuring on the circulation. It was possible to perform the measurement statically at 5 *strokes per minute*. The experiments performed after this showed that no hysteresis occurred at the different temperature levels either in *normothermia* or *hypothermia*. *It* proved possible without exception to eliminate static hysteresis by means of repeated measurements or preliminary expansion. Hence it must inevitably be assumed that static lung hysteresis is due chiefly to the difference in the number of open alveoli during inspiration and expiration at the corresponding volume level, as was pointed out by Mead *et al.* (1957) and Radford (1957). The static chest wall hysteresis (Tables 1, 4 and 5) did not change significantly during the present experiments. The change of this hysteresis was very small even in the measurements with cats no. 1, 2, 3, 4 and 6 (Table 4). The mean values of hysteresis of these cats were 0.48, 0.40 and 0.43 on lowering the temperature from 38°—30° down to 20°—10°C.

Comparative elastance studies conducted in normothermia and hypothermia showed that lung elastance did not change significantly when the temperature was lowered to 20°C. The same result had been achieved earlier by Radford and Frank (personal communication mentioned in Mead's review). Seehzer (1958) studied the variations of compliance in hypothermia, but he only took his observations down to 29°C. He too failed to observe significant changes. The changes in the elastance of the *chest wall in hypothermia, on the other hand,* have not been studied previously. In the present work it apparently rose *slightly when temperature* was lowered from 38°—36°C to 30°—28°. Experiments to gauge the effect of the weight of the surrounding water and ice, on the other hand, showed that the elastance increase could be attributed to the ice restricting the expansion of the chest (Figs. 16 and 17). This is supported by the observation that the elastance of the chest wall did not change significantly on lowering the temperature from 30°—28°C down to 20°—18°C (Tables 6 and 7).

The change from 20°—18°C down to 10°—8°C, however, did result in

VI. CONCLUSIONS

The author arrives at the following conclusions concerning the questions posed The conclusions are based on his own investigation results and observations in the literature

1 The difference in the number of open alveoli during inspiration and expiration at the corresponding volume level is the principal reason for static lung hysteresis It proved possible without exception to eliminate hysteresis by means of repeated measurements or preliminary expansion once the part of air leakage had been eliminated by adopting a technique which reduced it to a constant in comparative measurements

2 The hysteresis studies made in normothermia and hypothermia led to the same result The static lung hysteresis disappeared after the lungs were expanded by the 200 ml stroke before the measuring of hysteresis and this hysteresis was studied at one and the same volume level during inspiration and expiration The static chest wall hysteresis was very small in these experiments

3 No significant changes occurred in lung elastance when the temperature was lowered from 38°—36°C to 20°—18°C On moving down to the 10°—8°C level the elastance increase on the contrary was very highly significant This rise can be attributed to the increase in the opening pressure caused by the increase in surface tension and viscosity and the changes in pulmonary circulation although the effect of the lung tissue itself on the changes caused by the cooling process cannot be assessed from the present study The increase in the elastance of the chest wall when the temperature was lowered from 38°—36°C to 30°—28°C *can be explained by the weight of the ice* The distinct very highly significant increase when the temperature is lowered further to 10°—8°C is probably due to the effect of the low temperature on the chest wall

4 Air leakage from the lungs especially when great quantities of air are forced into them is a manifest factor distorting the interpretation of the results Therefore it is very important to pay enough attention to the selection of the method of measurement and of the equipment used for the investigation

opening pressure of the alveoli in fetal guinea pigs rose 30—50 per cent after the pulmonary blood vessels were ligated Jaykka (1957) established the significance of *'capillary erection" in the opening phenomenon of the alveoli in newborn It is known, furthermore, that surface tension and* particularly liquid viscosity increase in hypothermia (Seifritz 1952 Geigy 1960)

Both the present observations and those reported in the literature point to the fact that changes in lung elastance are at least to a great extent the result of steadily rising opening pressure of air spaces If elevated surface tension and liquid viscosity and the probable emptying of the capillaries *in the atelectatic areas result in the complete closure of the corresponding* alveoli, these atelectatic areas will not open in spite of repeated expansions Although the contribution of the changes in the lung tissue itself remains unclarified, their role can probably be thought to be considerably smaller, judging by the above findings

It was shown by Hajdu and O Sullivan (1950) that when raising the temperature from 0°C the work and tension of the contracting sartorius muscle of the frog increases reaching maximum at about 10°C Both are still nearly maximal at 15°C Then they drop on further increasing the temperature, first gently later more abruptly (Hajdu 1950) The increase in the elastance of the chest wall in the present work when the temperature was lowered to 10—8°C is probably due to the effect of cold on muscle tissue

mated without any exceptions by repeated measurements or by re expansion of the lungs both in normo and hypothermia The input and output pressures were equal at the corresponding volume levels The conclusion drawn was that the difference in the number of the open alveoli during inspiration and expiration was in fact the principal cause of static hysteresis

The changes in the static elastance values for the chest wall and the lungs were very highly significant when the temperature was lowered from 20°—18°C down to 10°—8°C The analysis of these changes are based on the results obtained in this work and observations in the literature

The leakage of air from the lungs and possibly also from different apparatus had a significant influence on the interpretation of the results, unless taken into consideration in the planning stage and in choosing the method

VII. SUMMARY

Changes in the static elastance *and hysteresis of the chest wall and the* lungs were studied in normo and hypothermia The elastance measurements were performed at 38°—36°C, 30°—28°C, 20°—18°C and 10°—8°C The hysteresis of the chest wall and the lungs were measured at 38°—30°C, 30°—20°C and 20°—10°C

Experiments were performed on 40 mongrel cats weighing between 1 9 and 3 0 kg (B_1 15 cats B 10 cats, B_3 15 cats) An additional 10 cats and 20 rabbits were used in advance to develop the method used in this work for comparative studies in normo and hypothermia The normothermia values of each subject form a control group The cats were anesthetized with sodium pentobarbitone intraperitoneally (40 mg/kg) and the rabbits with urethane *intravenously (0 3—0 4 g/kg) Suxamethonium* chloride, in small repeated doses of 25—50 mg was used to relax the muscles during the experiments

The static elastance and hysteresis measurements were performed ac*cording to the method developed by the present author The lungs were* expanded before the measurement in order to eliminate the effect of time The cats were kept in the same supine position throughout the experiment The pleural pressure measurement was employed The pleural catheter was at the same point during an experiment The values compared for normo and hypothermia measurements were always for the same animal

The measuring time of hysteresis could influence the results likewise the position of the stroke volume regulator of the respirator For this reason a part of the measurements of the static hysteresis was conducted at a single volume level only The measuring time was thus as short as possible In these cases the lungs *were expanded by closing the respirator* expiration tube for the duration of 3—4 strokes The part of air leakage had been eliminated by adopting a technique which reduced it to a constant in comparative measurements *The pressures measured at the same volume* level during *inspiration and expiration are thus really comparable*

The present work displayed that the static hysteresis could be elim

mated without any exceptions by repeated measurements or by re expansion of the lungs both in normo and hypothermia The input and output pressures were equal at the corresponding volume levels The conclusion drawn was that the difference in the number of the open alveoli during inspiration and expiration was in fact the principal cause of static hysteresis

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Page 14, line 23, for GURDIJAN, EVANS & LISSNER (1959) read LISSNER and GURDJIAN (1961)

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Page 43 Fig 15 line 5, for discharded read discarded.

Page 47, line 4, for strereocomparator read stereocomparator

Page 47, line 5 for disigned read designed

Page 72, line 13, for a present read at present

Page 73 line 4, for VON BAHR read VON BAHR and ERIKSSON (1961)

Page 75, line 18, for interrection read interreaction

Page 76, kp, for correspondings read corresponding

Page 77 line 3, read ALDMAN, B and SIGMARK, E Photogrammetric method for etc

Page 78 bottom, add, LINDGREN, S Personal communication, 1961

Page 80 below line 13, add, STAPP, J P and ENFIELD, D L · Evaluation of the lap-type automobile safety belt with reference to human tolerance Paper presented at SAE summer meeting, Atlantic City N J, U S A 1958

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CONTENTS

ACKNOWLEDGEMENTS

This investigation deals with physiological problems which involve many aspects of a purely physical nature It is a pleasure to record my sincere gratitude to Professor U S VON EULER for stimulating advice and constant encouragement so necessary in this borderline area of research I am also deeply indebted to Professor E LINDGREN whose never failing interest and personal influence made the roentgen studies possible

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INTRODUCTION

The high frequency of crashes has made imperative the development of methods for decelerating the occupant of automotive vehicles in the most effective way possible by proper restraint The human body can probably be effectively protected from injuries arising from mechanical force, by submersion in a resistant water tank (DE HAVEN 1944, CLARK 1961, GRAY 1961), which is the principle of protection in prenatal life But this method is not applicable to active people in daily traffic For this purpose the method of protection must be as simple as possible and yet effective

If an occupant is to incur the cost and inconvenience of using a means of protection it must be effective in most accidents, also in city traffic For even in a crash at low speed the change in the velocity of the vehicle may be such that the occupant will not be able to retain his place in the vehicle but will impact its interior or be thrown out of it A shock-absorbing device to cushion the secondary impact can be made both as an improvement of the shock absorbing properties outside and inside the vehicle (RYAN 1960) and as a restraint for the occupant which may even prevent the secondary impact

If a passenger is to be protected from hitting the frontal limitation of the passenger compartment in a collision by a body restraint that would also prevent ejection this can be achieved by applying one of the two following methods

I If the space in front of the occupant is large enough, a simple lap restraint that allows the occupant to jack-knife over the belt may be effective (STAPP *et al* 1957), if the stretching of the strap is sufficiently limited to prevent contact with frontal structures This method thus requires the use of *short stretching straps*

II If on the other hand the space in front of the occupant is not large enough to allow jack-knifing without impact with the frontal structure, the occupant must be decelerated in an upright sitting posture This requires the *use of an upper torso* restraint in combination with the lap restraint But the construction of the upper-torso is different from that of the pelvis and there are reasons for believing that the critical velocity for deformation of the upper torso is much lower than for the pelvis If that is the case it would

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Stockholm, April 1962

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BACKGROUND TO PRESENT INVESTIGATION

EXPERIMENTAL STUDIES

It has long been known that human tolerance to accelerations during longer periods is limited by a shift in body fluids that can only partly be prevented by special devices (G-suits, body posture etc) In a paper of 1957 STAPP states Prolonged decelerations in which durations range from 2 to 3 second demonstrate that hydrostatic pressure effects due to displacement of body fluids have a latent period of 2 second before they are appreciable and that they are definitely evident at 4 to 6 second of exposure to as low as 10 g at 500 g per second rate of application" It has also been demonstrated, for instance after falls from high altitude, that human tolerance is much higher if the time for deceleration is sufficiently limited (DE HAVEN 1944) Much experimental research of importance has been done in this field

STAPP (1949) used a linear decelerator to expose human volunteers to decelerations from back to chest in the seated position up to 30 G Total duration of exposure ranged from 0 15 to 0 42 second He states that 'Ultimate decelerations voluntarily tolerable to the subjects used was not reached since the tests were halted to improve the mechanical reliability of the decelerator"

In 51 experiments the subject sat facing forward on an improved decelerator in the range of deceleration from 10 to 45 4 G The duration of deceleration ranged from 0 15 to 0 35 second in these experiments In a report from 1951, Stapp states 'Subjectively limits of voluntary tolerance were approached at 17 0 G at 1 000 G per second rate of onset with the standard Air Force harness configuration at 38 0 G at 1,350 G per second with the inverted V leg strap added to the shoulder straps and lap belt assembly and at about 46 0 G with rate of change of deceleration of about 500 G per second, using the latter configuration Much higher levels can be survived, although reversible injurious effects may intervene'

These experiments were made to establish human criteria for improvements in aircraft safety But STAPP also made and contributed to several tests for the evaluation of the lap-type automobile safety belt (STAPP *et al* 1957)

be desirable to attain a lower rate of onset of the force, created by inertia during deceleration, against an upper torso Such a lower rate of onset can be achieved either by using shock-absorbing material to decrease the deceleration of the vehicle, shock absorbing anchorage for the restraint or simply by using *long-stretching straps*

Thus it appears to be a question of primary importance what stretching properties the restraint should have in order to provide maximum security for the occupants Great difficulties are involved in the study of the effect of high peaks of deceleration with a duration of less than 20 msec To a great extent such peaks are damped out in the restraint but under critical conditions they may rupture the material and it is therefore important to find methods of studying the effect of such peaks in different materials used for crash-survival devices

As no investigation has been carried out and reported in the literature where sufficiently accurate recording methods for such a study have been used, the present investigation was planned and started with the primary object of finding methods of recording the effect of short-term, high peaks of deceleration This primary object can be reached by answering the following questions

1 Is it possible to record the effect of peak decelerations with a duration of 20 msec or less with sufficient accuracy?

2 What deformation could be recorded in the body restraint subjected to such peak decelerations?

3 How can the displacement and deformation of vital parts of the body decelerating under these conditions be studied?

4 What are the limiting factors for the effectiveness of a body restraint under such conditions?

The answer to these questions should be of interest not only for the standardization and production of safety devices for automotive vehicles but also for many branches of medical science, as being basic to the study of the biodynamics of trauma

The aim of this publication is to give a survey of the recording methods with special reference to the recording with high accuracy of rapid deformative movements and the results of investigations where these methods have been used for the study of long-stretching body restraints such as the automobile safety belt

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Different types of decelerators were used for these experiments, viz swing seats and catapults with different types of brakes In a report of 1957 Stapp states "Human tolerance to deceleration with a lap-belt restraint three inches wide was not exceeded in these experiments Twenty-three G at the lap-belt area at a rate of onset of 570 G/sec and a duration of 0 001 second can be sustained without injury" Contusions and abdominal-muscle soreness followed impact load of 13 G at 300 G per second for a duration of 0 002 second Back-muscle soreness can occur following 26 G, 850 G per second with a duration of 0 002 second

In 1947 a committee was appointed by the President of Cornell University to study transportation safety, the Automotive Crash Injury Research at the Cornell Medical College Group in New York City The automotive industry also formed research groups (General Motors Chayne Ford Motor Company Haynes, Fredericks, Ruby etc) and the Institute of Transportation and Traffic Engineering, University of California (ITTE-UCLA), Los Angeles, carried out car-to-car and car-barrier impact full-scale crash investigations on an experimental basis (MATHEWSON & SEVERY 1956—1961)

During the last decade much work to obtain data on human tolerance to rapidly applied accelerations has been done by the US Air Force, the Public Health Service, the NASA, and various universities in the United States (LISSNER 1961)

During recent years impact studies have also been made in other countries for instance in Britain by the Road Research Laboratories and the RAF and in Germany by different manufacturers, but these tests have been only briefly reported in the literature (STARKS 1960 GUINARD 1961)

THEORIES ON HUMAN TOLERANCE

Improvement of the crashworthiness of vehicles is highly desirable, but will not sufficiently increase the safety for occupants of vehicles already on the road Therefore a shock-absorbing restraint is the solution of the problem in all vehicles where such a device can be used, because it provides a possibility of making use of the protective capacity of the vehicle The automobile safety belt is a step in that direction and many different kinds of such belts have been produced during recent years The problems concerning their effectiveness may be representative of the whole field of crash survival research It is not difficult to judge whether these belts are simple to use or not but the question whether they are effective in case of an accident has given rise to much discussion The reason for this discussion is that occupants are not injured in all accidents Even in the most violent types of accidents it

sometimes happens that one or more occupants sustain only minor injuries or are even uninjured But the risk of being injured is high enough to make imperative extensive studies of which are the most dangerous accidents and of the best means, first of preventing them and secondly, of protecting occupants of vehicles involved in these accidents

Statistical analysis has shown that the risk of being injured is higher when an occupant is ejected from a car (WOLF *et al* 1961) On the other hand it is obvious that in specific cases it might be even more dangerous for the occupant to remain in the vehicle However, in "ordinary" accidents this is very seldom the case

There are in accident material so many variables to be checked that a series would need to be extremely large to make possible an examination of the effect of a change in one single factor, such as a certain type of body restraint, in different types of accidents Therefore many investigators have preferred to study the problem experimentally The type of accident that for technical reasons can be expected to be the most serious and yet where means of protection can be applied most easily, for instance by the use of a body restraint, is the head-on collision All investigators agree that the head on collision is a serious accident even at low speed The reason for this is that the speed of the vehicle is changed so suddenly that the occupant cannot follow it, but will be thrown against the interior of the vehicle The change of speed may then be still higher for the occupant

A U S National Safety Council release on urban automobile accidents shows that 40 per cent of fatalities were associated with speed of 20 mph or less while 70 per cent of fatalities were associated with speeds of 30 mph or less (DE HAVEN 1944)

The risk of being lethally injured when impacting a hard surface has been estimated to be 50 per cent at an impact velocity of 27 ft/sec or about 8 m/sec (WHITE 1960)

Different opinions exist about the decisive physical quantity in judging impact injuries and breakage of material A complete judgment can be made only with a complete knowledge of the total time course of the force acting on the object and the characteristics of that object, i e the reaction of the object to a given force time pattern

The primarily measured quantity is seldom the force as a function of time but rather the deceleration or the displacement as a function of time, from which the time course of the force is derived by the mechanical laws of motion However this quantity usually is a complicated function of time (for instance with short term peaks) and a simpler primary quantity is therefore desired Maximal deceleration has thus often been considered a decisive quantity The total velocity change and sometimes the time derivative

of deceleration have also been used for that purpose Though there are situations where maximal deceleration or acceleration may be decisive (for instance for some injuries to people exposed to acceleration during long periods, as in the launching period or the re-entry to the athmosphere in manned space flight) and other situations where the total velocity change seems to be decisive (for instance some injuries to people falling from high altitudes) no general rule can be given for the judgment of the decisive quantity for damage in complicated structures The reason for this is that — for instance for the human body — different kinds of injury caused by the same impact may be referred to different decisive quantities of the impact pattern Skeletal parts, as for instance the skull, are more sensitive to short-term accelerations than are softer parts, such as the heart Therefore it is necessary to measure the total force-time relation (or some other quantity from which this relation can be calculated) with sufficient accuracy to enable the necessary simplifications at a later analysis of the risk of injury for different objects

In the literature, the difference in principle between short stretching and long-stretching straps has been very little discussed STAPP (1958) states that "Elastic stretch in the restraint system should be eliminated, in so far as possible", and "A drowing type or plastically deforming force attenuating device should be *incorporated* in a restraint system to limit peak loading force"

KORNHAUSER (1958) CHAPLAND GELL HOLCOMB and PAYNE (1961) have discussed the problem whether there exists a definite limit between short-term and long-term deceleration that would make a comparison between, for instance, "ordinary" car accidents and some types of aircraft accidents impossible because the cause of injury would be referred to different decisive physical quantities They state that from a theoretical point of view assuming *an optimal distribution of the forces* loading the body the duration of acceleration is of significance for human tolerance By studying a mass-spring system KORNHAUSER was able to distinguish between three different "zones of impact" (KORNHAUSER 1961)

1 Short duration impact zone is characterized by $0 < \frac{t_I}{T_n} < 0.4$

where t_I = duration of impact, or rise time of pulse

T_n = natural period of vibration

In this zone onset rate, pulse shape or peak acceleration have negligible influences only velocity change is important

2 Intermediate duration impact zone $0.4 < \frac{t_I}{T_n} < 3$

Both onset rate and total pulse duration are important, with a spread factor of two in acceleration required to cause a given degree of damage at a fixed value of velocity change

3 Long duration acceleration zone $\frac{t_t}{T_n} > 3$

Onset rate is the primary influence here, with a spread of a factor of two between the slowly rising acceleration pulse and the infinite-onset rate pulse

The application of this theory to human strength can be done in the form of a so-called sensitivity curve (KORNHAUSER 1958, 1961) Such a curve is characterized by two asymptotes, one corresponding to the short-duration impact zone and the other to the long duration acceleration zone The criteria of damage in the short-duration impact zone is the velocity change 25 m/sec and in the long-duration zone the acceleration 20 g The nature of the sensitivity curve is such that both these values must be exceeded concurrently for damage to occur The figures given refer to a well-supported human being in the supine position

'Corner duration", i e the duration of the intermediate-duration impact zone in this sensitivity curve, is about 0 12 second

On the other hand, STAPP and others have found the problem more complicated from a practical point of veiw STAPP (1961) states

'In all cases, the effects result from an interaction of linear, quadratic and cubic factors of motion with inertial factors complicated by reasonant modes, elastic deformation, viscous damping, and structural complexity In terms of the fundamental concepts of time and distance, the motion factors are defined as time-distance derivatives

Where $l =$ distance, and $t =$ time

$l/t =$ velocity

$l/t^2 =$ acceleration, or velocity change with time in either rate or direction

$l/t^3 =$ jolt, or rate of change of acceleration

Mass set into motion has corresponding inertial derivatives

Where $m =$ mass, $l =$ distance, and $t =$ time

$ml/t =$ momentum, $ml^2/t =$ action

$ml/t^2 =$ force, $ml^2/t^2 =$ work, energy

$ml/t^3 =$ onset, $ml^2/t^3 =$ power

A body having elastic deformation will have two components of reaction to being set in motion (1) a constant quantity corresponding to the static force required to displace the mass through a given distance, known as the forced response and (2) a variable quantity relating to force applied to the elastic deformation, known as the free response The sum of the forced response and the free response is the dynamic response, for a given rate and duration of applying a force or load, there will be a maximum dynamic response which is a function of the natural frequency and is called the dynamic load factor"

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perties of the body of the car and of the occupant might also cause such time lags in the acceleration of mass elements That means *inter alia* that using a short-stretching restraint might imply participating in the least part of deceleration of the vehicle The deceleration pattern of the car is of importance in this connection The use of a long-stretching restraint, on the other hand might imply a deceleration that is governed mainly by the stretching properties of the restraint Between these two extremes all variations are possible

The use of a long-stretching restraint will therefore make it less important to know in detail the deceleration pattern of the vehicle, in other words the long-stretching restraint will be more useful for any kind of vehicle, if only the restraint is tested and found to have a high enough critical velocity for rupture of the material In some accidents it might also imply a higher peak deceleration for the occupant than for the car, this will happen also if a short-stretching restraint is not extremely well adjusted to the occupant The maximum rate of onset of deceleration for the restrained object will then be governed mainly by the stretching properties of the straps Therefore experiments with long-stretching restraints ought to be carried out by imposing a very quick stop of the anchoring point so that the worst possible conditions are applied to the system.

RECORDING METHODS

For a study on the dynamic deformation of the restraint system it is necessary to have recording methods with sufficient accuracy to record the effects of deceleration peaks of short duration (10—20 msec.)

Many difficult problems are involved in the recording of events taking place in complicated mechanical structures in such a short time The risk of total recording failure is also appreciable when forces of thousands of kiloponds are released in so short a time Therefore many different recording systems have been tested in earlier investigations

Accelerometers of mechanical strain-gauge, differential transformer or piezo-electric types have been used as detector units to record the deceleration in various parts of the vehicles and on dummies, experimental animals or human volunteers GERLOUGH (1954) has discussed the difficulty of distinguishing small displacements of vibrating members from large accelerations In order to reject these vibrations he recommends the use of accelerometers with a natural frequency of 60—70 cycles per second or a low pass filter in the circuit In order to get useful results from the accelerometers readings, most investigators seem to have used accelerometers with natural frequencies of less than 100 cycles per second.

He further states that "In system of many degrees of freedom, dynamic load factors can vary with location in the structure and may considerably exceed the value of 2.0." STAPP (1961) notes that impedance values for vibrations applied transversely to the long axis of the human body are not available. Data on resonant response of suspended organ masses within the body, such as the heart, are also lacking (GOLDMAN and v. GIERKE 1960).

Although STAPP has reported a latent period of about 0.2 second for the appearance of hydrostatic effects after abrupt application of pressure to the chest, he also states (1961) that "Mild transient cardiovascular reaction has been observed at less than 25 G peaks where duration was less than 0.1 second, and quite severe cardiovascular shock *resulted from application* of 38.6 G at 1,370 G per second rate of onset for 0.12 second total duration, which corresponds to 8—9 cycles *per second resonant frequency*. The severest and most persistent signs of shock were observed in a backward facing deceleration during which Captain Eli Beeding was exposed to 2,139 G per second to a peak of 40.4 G for a duration of 0.040 second. This corresponds to 12—14 cycles per second. The dynamic response to this impact was a peak measured on the sternum of 82.6 G at 3,826 G per second — approximately double the rate of onset and magnitude of the applied impact — which corresponds to about 12 cycles per second of resonant response. The impact force was apparently amplified with a dynamic load factor of 2.0 by the elastic response of the rib cage."

GURDIJAN, EVANS & LISSNER (1959) have, in their experimental head impact studies, been able to demonstrate that the appearance of skull fracture and concussion can be referred to certain physical quantities. If that is also true for the rest of the body it may explain why such widely differing figures have been found for biological tolerance in experimental work.

With the exception of NICHOLS's (1954) work on dynamic response of restrained objects, which was started in cooperation with STAPP, very little has been reported about the stretching properties under dynamic conditions of the restraint material used in different experiments, even in NICHOLS's report the studies are limited to a straight length of a simple strap and the stretching is only studied during long-duration decelerations. CLARK (1961) states that body distortion is the cause of injury arising from acceleration stress.

When judging to what deceleration zone (KORNHAUSER 1961) a restrained object should be referred, it is important to know that from several experiments there is reported a time lag between the beginning of the deceleration of the vehicle and the beginning of the deceleration of the restrained object (STAPP 1957, SEVERY 1959). This time lag is mainly caused by a slack in the restraint that is unavoidable in practical use (RYAN 1960), but the elasic pro-

perties of the body of the car and of the occupant might also cause such time lags in the acceleration of mass elements That means *inter alia* that using a short-stretching restraint might imply participating in the least part of deceleration of the vehicle The deceleration pattern of the car is of importance in this connection The use of a long-stretching restraint, on the other hand might imply a deceleration that is governed mainly by the stretching properties of the restraint Between these two extremes all variations are possible

The use of a long-stretching restraint will therefore make it less important to know in detail the deceleration pattern of the vehicle, in other words the long stretching restraint will be more useful for any kind of vehicle, if only the restraint is tested and found to have a high enough critical velocity for rupture of the material In some accidents it might also imply a higher peak deceleration for the occupant than for the car, this will happen also if a short-stretching restraint is not extremely well adjusted to the occupant The maximum rate of onset of deceleration for the restrained object will then be governed mainly by the stretching properties of the straps Therefore experiments with long-stretching restraints ought to be carried out by imposing a very quick stop of the anchoring point so that the worst possible conditions are applied to the system

RECORDING METHODS

For a study on the dynamic deformation of the restraint system it is necessary to have recording methods with sufficient accuracy to record the effects of deceleration peaks of short duration, (10—20 msec)

Many difficult problems are involved in the recording of events taking place in complicated mechanical structures in such a short time The risk of total recording failure is also appreciable when forces of thousands of kiloponds are released in so short a time Therefore many different recording systems have been tested in earlier investigations

Accelerometers of mechanical, strain-gauge, differential transformer or piezo-electric types have been used as detector units to record the deceleration in various parts of the vehicles and on dummies, experimental animals or human volunteers GERLOUGH (*1954*) has discussed the difficulty of distinguishing small displacements of vibrating members from large accelerations In order to reject these vibrations he recommends the use of accelerometers with a natural frequency of 60—70 *cycles* per second or a low pass filter in the circuit In order to get useful results from the accelerometers readings, most investigators seem to have used accelerometers with natural frequencies of less than 100 cycles per second.

He further states that "In system of many degrees of freedom, dynamic load factors can vary with location in the structure and may considerably exceed the value of 2.0." STAPP (1961) notes that impedance values for vibrations applied transversely to the long axis of the human body are not available. Data on resonant response of suspended organ masses within the body, such as the heart, are also lacking (GOLDMAN and v. GIERKE 1960).

Although STAPP has reported a latent period of about 0.2 second for the appearance of hydrostatic effects after abrupt application of pressure to the chest, he also states (1961) that "Mild transient cardiovascular reaction has been observed at less than 25 G peaks where duration was less than 0.1 second, and quite severe cardiovascular shock resulted from application of 38.6 G at 1,370 G per second rate of onset for 0.12 second total duration, which corresponds to 8—9 cycles per second resonant frequency. The severest and most persistent signs of shock were observed in a backward-facing deceleration during which Captain Eli Beeding was exposed to 2,139 G per second to a peak of 40.4 G for a duration of 0.040 second. This corresponds to 12—14 cycles per second. The dynamic response to this impact was a peak measured on the sternum of 82.6 G at 3,826 G per second — approximately double the rate of onset and magnitude of the applied impact — which corresponds to about 12 cycles per second of resonant response. The impact force was apparently amplified with a dynamic load factor of 2.0 by the elastic response of the rib cage."

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ANALYSIS OF RECORDING METHODS USED IN EARLIER INVESTIGATIONS

Almost all investigators point out the difficulties in recording movements, velocities, decelerations and forces in this kind of experimental work In order to minimize the risk of failure in the recording of such quantities in costly investigations, most workers have used several of these recording methods at the same time Results from different recording methods have also been compared as a check on the accuracy of the different methods

High-Speed Cinematography

As mentioned before, several authors express uneasiness as to the accuracy of the accelerometer readings The necessity of lowering the natural frequency of the transducers has led some investigators to use results from the high-speed films as a control of these readings SEVERY and BARBOUR in 1956 published an analysis of the acceleration accuracy of the high-speed camera film in which they claim that a frame by-frame analysis of these films will provide an extremely high accuracy Their list of sources of error contain only these three items time measurements, distance measurement and plotting and curve-fitting error

Before this list is discussed it is important to describe how the high-speed cameras have been used during the present experiments Usually the cameras have been placed at a distance of about 60 feet from and at right angles to the moving direction of the vehicle so that it would be possible to see the collision centre and the whole vehicle at the same time during impact The shutter speed has often been of the order of 1000 frames per second and the speed of the car of the order of 20—25 mph (SEVERY and MATHEWSON 1956) or higher Converting these figures into millimetres, we get approximately

Distance 18,000 mm Picture field 6,000 mm.

Impact speed 10 mm per msec

Holcomb (1961) has criticized the use of accelerometers on the human body because it is not possible to avoid disturbances in the readings arising from the fact that the body is deformed, which may cause tilting of the accelerometers, and in view of the fact that an accelerometer mounted on a deforming surface may constitute its own mass-spring system the output as read on the accelerometer then has little relationship to the input

High-speed cameras, ordinary cinematograph cameras and still cameras have been used to record complete movements of the riders and the vehicles during deceleration Synchronization of the different recording methods has also been used In some experiments a great number of different cameras have been used simultaneously, as for instance in the ITTE-UCLA (p 10) tests

Electrical or optical methods for recording the speed of the object have been necessary in some experiments Magnetic pick-ups, photo-cells, Berkeley Counters and different kinds of triggering devices have been combined with the above-mentioned recording methods

The impulses from the transducers have been fed to the recording instruments either directly by cables or by telemetering systems

Concerning inaccurate determination of the time as a source of error, these authors say that it is most convenient to use a timing lamp marking the edge of the film It appears from the text that they have used a frequency for that lamp of 120 marks per second and it is stated that "the film speed was found to increase linearly over the section in consideration" From other publications it appears that the speed does not increase linearly over a longer period (PALMER 1958) and therefore the statement in the list of errors "the slopes and therefore the magnitude of the resulting velocity and acceleration have not been altered" only holds for short parts of one film and uniform accelerations of film and object In later experiments SEVERY has used an additional timing device in the picture field in order to correlate films from different cameras

About distance measurement SEVERY and BARBOUR note that "two times the standard deviation of the measurements was determined to be in the order of ±0 0005 in" Furthermore these authors state that "Although amplified to the extreme, the errors in deceleration did not vary from the observed values more than about 13 %"

The experiment carried out by SEVERY and BARBOUR to estimate the acceleration accuracy, using a falling sphere, is representative only for cases where it is known or supposed to be known that the acceleration is constant There the standard error is a constant In collision tests, where the acceleration is not independent of time but can be representated by a polynomial of higher degree, the standard error is not a constant but can be calculated with the aid of the law of error propagation

To demonstrate this, data derived from the velocity-time plot in Fig 4 of SEVERY and BARBOUR's paper can be used From the general distribution of the plotted observations it seems likely that a systematic *error is present* in the measurements Such a systematic error might be the result of different stretching of different parts of the film during exposure and might be serious if only a small part of the film is used for calculation Compare, for instance the distribution of observations between the time marks of 80 — 100 msec with the least-squares fit for the whole time course To give comparable figures, as some errors might derive from the reproduction of the plot, the true error assuming a constant acceleration is given

The slope of the line corresponded to an acceleration of 34 17 feet/sec² (10 42 m/sec²) The correct value corresponded to 9 791 m/sec², the true error thus being + 0 63 m/sec² or 6 4 % However, it is possible to estimate the standard error of unit weight by taking the differences between the measured values of velocity, and the smoothed velocity curve (the straight line) in the figure This gives

$$M = \pm\sqrt{\frac{[\Delta\Delta]}{n-2}}$$

The High-Speed Camera

In the high-speed camera the film is driven through the camera at considerable speed. Usually one motor drives the drive sprocket and the same or a separate motor the take-up spool (PALMER 1958). This mechanism may cause some stretching of the film before exposure. Usually only fresh film can be used in these cameras, because if the film has been allowed to dry it will have lost its elasticity and will break when run through the camera. Thus, stretching of the film will occur and the subsequent shrinkage during development and drying may then give a different scale for measurements in horizontal and vertical direction and even in different parts of the film. Testing the camera for use in investigations in which displacement readings are made in the horizontal direction by photographing a falling body and taking readings in the vertical direction, as described by SEVERY and BARBOUR, will therefore be of limited value.

The shutter in the high-speed camera is of a special design. As this type of cameras uses a continuously moving film, the image is made reasonably stable in relation to the film by means of a parallel-sided prism rotating between the objective lens and the film plane. All objective lenses cause some degree of distortion to the image. In these cameras consecutive frames will not be exposed through identical optical systems because they are exposed through different sides of the rotating prism. Therefore the distortion may vary from frame to frame. The use of rotating prism to stabilize the image of the film during exposure involves two problems which may influence the accuracy of the recording: one is the difficulty of synchronizing image and film speed during a period sufficiently long for exposure, and the other is the varying distance from the optical centre of the objective lens to the film plane in different positions of the prism during exposure. The solution of these problems must be a compromise between accuracy and exposure time, and it will therefore be necessary to test each camera used for measuring purposes for image distortion at the different shutter speeds to be used during the experiments. The radial distortion can easily be measured by, for instance, HALLERT's grid method (HALLERT 1954—55).

Film Analysis

If the film is analysed through another optical system where readings are not made close to the optical axis the distortion of that system also must be known. It is possible but not certain that these sources of error are included in what SEVERY and BARBOUR call "the grain size of the film and other inherent sources of error".

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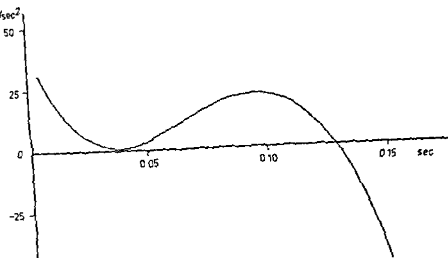


Fig 1 b

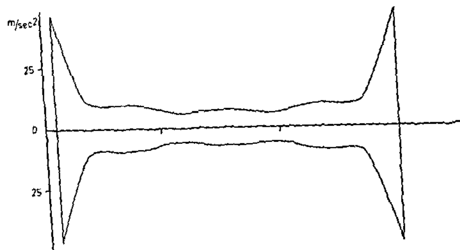


Fig 1 c

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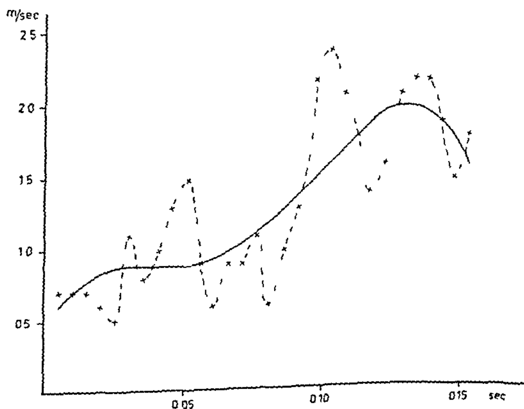


Fig 1 a

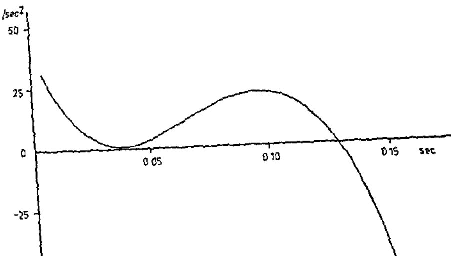


Fig 1 b

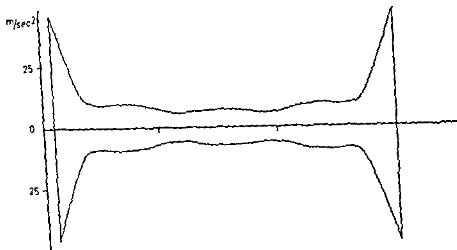


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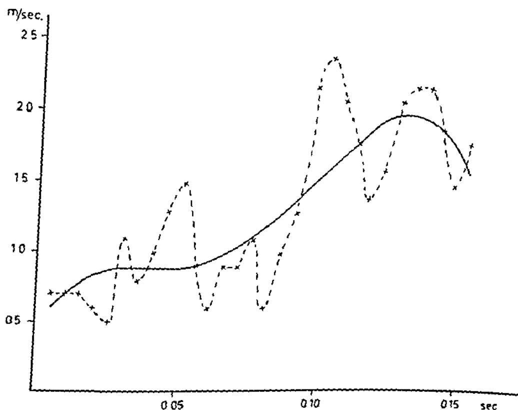


Fig. 1 a.

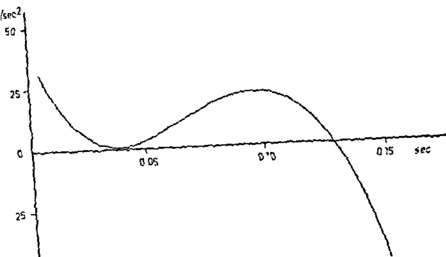


Fig 1 b

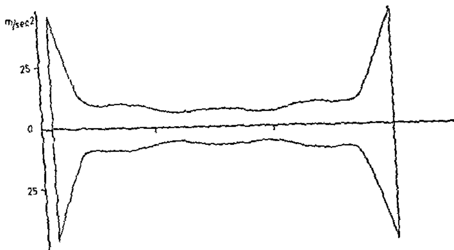


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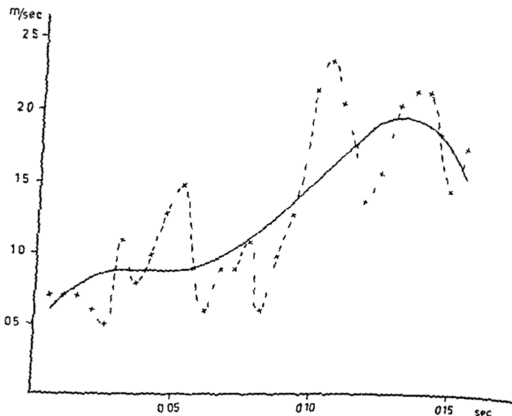


Fig. 1 a

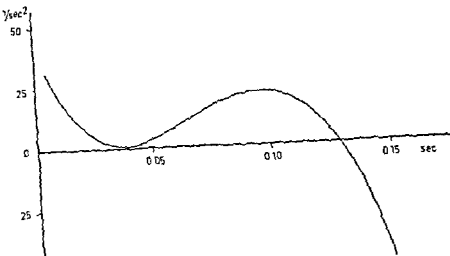


Fig 1 b

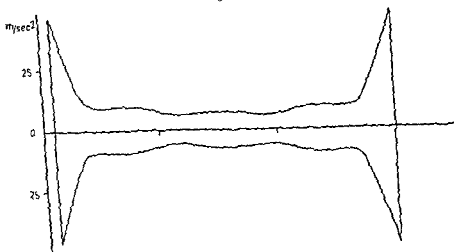


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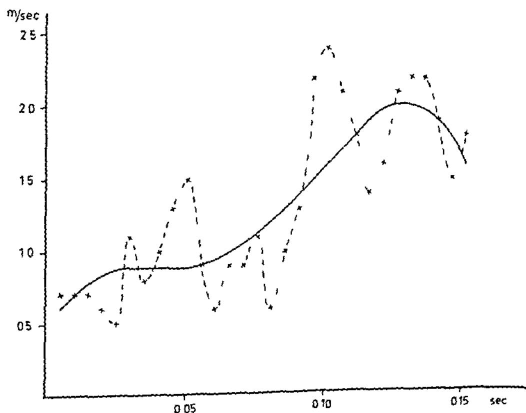


Fig 1 a

EXPERIMENTAL SECTION

INTRODUCTORY REMARKS

As early as the 1930's some attempts were made to use a kind of fastening device for drivers of racing cars The pioneers found it easier to drive a fast car if they were attached to the seat and so could feel the movements of the car better The idea that a link between the car and the driver would result in safer driving in ordinary traffic too is interesting, because the complex process of driving a car safely is not only the result of visual and acoustic stimuli but is also controlled by the sensory stimuli which give the driver information about how his vehicle is behaving under his guidance

In the 1940 s these early results were combined with the assumption that a safety belt could protect the driver from injuries in case of an accident And in the 1950 s the reports from several research activities in that field stimulated the production of various kinds of safety devices It became evident to the Swedish authorities that the automobile safety belt could offer some protection to car occupants in traffic accidents Some tests were made by the Swedish Power Board (ODELGARD & WEMAN 1957), these confirmed the earlier results but also indicated that many car safety devices produced at that time in Sweden were of rather poor quality

A close cooperation was then established between the responsible authorities in this field The National Road Board the National Workers' Protection Board the Medical Board and the Official Swedish Council on Road Safety Research decided that the problem should be scientifically investigated and that a national standard should be set up for automobile safety belts At the beginning of 1957 the author started investigating the protective capacity of various types of safety belts with a grant from the Council on Road Safety Research The first Regulations for Automobile Safety Belts" were issued by the National Road Board in April 1958 and it was stated that these regulations would be revised if the results of the investigations made it desirable A partly revised edition of the standard will then be published by the Road Board

acceleration It is observed that even in the best part of the acceleration time curve between 20 and 130 msec the variation from the correct value amounts to ± 100 per cent

As the results in this figure are derived from SEVERY and BARBOUR's data assuming the situation with the results from a collision test — that is, we do not really know what time function the acceleration represents — it is obviously necessary to analyse the accuracy of the measurements at every part of the curve and give these values together with the results In doing so we shall find that high speed photography is not so accurate a method for determining the acceleration pattern in collision tests, even with a frame-by-frame analysis of the film, as it has been considered It is also obvious that any uneven stretching of the film will not be detected when a timing lamp with a frenquency of 120 marks per second is used and therefore a higher *frequency of the timing light should be used in order to control errors from* this source in the time measurements This is of importance also because the acceleration pattern from high speed film analysis has sometimes been used in judging the natural frequency of the accelerometers used in collision tests (GERLOUGH 1954)

be in the order of 100 g That situation, with sufficient margin, could probably be representative for a head-on collision taking place even at a higher speed It now became necessary to find a method of recording the movements in space of the anchoring points with very high accuracy in order to make possible a study of the stretching process in the strap Experiments were made on single straps of different kinds and with complete safety belts The complete belts were tested using dummies of different kinds and it was of great interest to find out to what extent the human body would load the different straps and whether the situation was different for the driver and the passengers Figures have been published showing that in an accident there is a different risk for the driver, the front seat passenger and the rear-seat passengers (WOLF *et al* 1961) What could be the cause of such a difference?

One factor might be that during most accidents of the type in question the front seat is torn loose and slides forwards, decreasing the space and increasing the weight to be stopped for the front-seat passengers whereas the rear-seat less often behaves in that way The result is an increasing space for the rear-seat passenger, who will hit a limitation which may still be moving forwards The driver, who is usually aware of the coming accident, will probably have his hands resting on the steering wheel his feet on the pedals at the moment of collision That might reduce the weight to be stopped against the frontal limitation of the compartment Thus it became interesting to study if the contact with the car through the floor or a steering wheel could reduce the demand on the belt

When these problems had been solved to some extent, it was possible to study the response of the human body to the forces These problems could be studied in two different ways One way is to record the movements of anthropometric dummies experimental animals and human volunteers, the other way is to study real accidents where safety belts had been used

When the vehicle is suddenly stopped, a strain wave starting from the anchoring point will spread along the strap, being reflected at the end points and passing along the strap several times during the period of elongation This means that the elongation of the strap will not be of the same magnitude at different parts of the strap at the same moment This phenomenon must be considered when the dynamically measured force-strain curve is plotted The velocity of the strain wave is limited, which means that if the relative collision velocity is higher than the velocity of the wave and the deceleration period is short compared with the time the wave takes to travel over the length of the strap the strap will break independently of the force produced by the inertia of the body In fact this can happen at a much lower velocity, i e the critical velocity for that strap In some of the straps the velocity of the strain wave was about 250 m/sec, the critical veloc[illegible]

Already from the beginning it was obvious that the limited space in European-built cars made it necessary to have an upper-torso restraint if the passengers were to be protected from injuries to head and thorax in accidents The average distance for deceleration of the front-seat passenger in cars registered in Sweden is about 45 cm (25—65 cm) An investigation of the Scandinavian Surgical Association 1957 had confirmed earlier results (BRAUNSTEIN 1957) that injuries to head and thorax are the predominating cause of death for car passengers Two main problems then arose Would an upper-torso restraint cause injuries to the thorax or to the shoulders during deceleration? Would it cause whip-lash injuries?

As the problems seemed to be connected not only with the peak force on the body but also with the rate of onset or the duration of that force, it was necessary to study the stretching properties of the belt But these would be dependent on the deceleration of the car, as the anchoring points in the car would be the place where the loading force was applied to the straps The rate of onset or the duration of that force, on the other hand, might be of significance for the stretching of the belt

Thus it seemed necessary to make a study of the deceleration during an accident of those parts of European cars where anchoring points could be placed The investigation should be detailed enough to reveal the rate of onset and the duration of the deceleration, but could be limited to the worst possible situations, as it would neither be possible nor of much interest to study the best possible situation This deceleration could then be repeated in laboratory tests, where the forces in the strap could be systematically investigated In such tests dummies, animals and human volunteers could be used

As safety belts were already being used at that time by some people it was urgent to find the most suitable anchoring points for the types of belts then being produced Of these the two main types were the single diagonal chest sash anchored in the doorpost and in the floor and the combined type with one lap strap and two shoulder straps all anchored at one point to the floor

The worst possible situation with respect to the force in the belt would be a head-on collision with a higher relative collision speed i e a large difference between the speed a moment before and a moment after the collision That situation would also be of most interest because the head-on collision represents an accident situation where the car itself could present the highest degree of protection to the passengers and the type of accident with the highest risk for serious injuries due to high relative impact speed

In the laboratory experiments an impact speed of 10 m/sec could easily be produced using a catapult with a falling weight With a total time of about one hundredth of a second for that change in speed the deceleration would

fluenced the planning of the investigation, as it made it desirable to collect useful data about the action of these belts as soon as possible At the beginning of 1958, therefore, a series of car barrier impact collisions were arranged in order to study the deformation pattern of European cars and the influence of safety belts on anthropometric dummies during impact

A dummy was built with a metal skeleton made of steel and aluminium A normal weight distribution was arranged by providing additional lead weights and a rubber sack, placed in the combined thoracic and abdominal cavity, which could be filled with various materials in order to obtain an appropriate centre of gravity for the torso (Fig 2)

The tests took place at Jordbro near Stockholm in the spring of 1958 Six European cars and one American car were crashed into a barrier consisting of stone blocks and sand and having a wooden front The cars were



Fig 2 Metal skeleton of the anthropometric dummy

Mobility in different joints

Joint / movement	Degrees
Lumbar spine	
Flexion	90°
Extention	30°
Lateral flexion	20°
Rotation	30°
Spine of the neck	
Flexion	30°
Extention	30°
Lateral flexion	40°
Rotation	unlimited
Shoulder	
Forward elevation	170°
Backward	40°
Adduction	30°
Abduction (incl clavicula)	150°
Rotation inward	40°
outward	40°
Elbow	
Flexion	150°
Supination	80°
Pronation	80°
Wrist	
Flexion	80°
Extention	70°
Hip	
Flexion	110°
Extention	20°
Adduction	20°
Abduction	40°
Knee	
Flexion	120°
Foot	
Dorsalflexion	20°
Plantar "	40°
Rotation (total) inward	40°
outward	50°

be about 50 m/sec , which will give a fair margin for use as automobile safety belts

It is reasonable to suppose that a critical velocity exists also for the deformation of various parts of the human body Therefore the stretching of the safety-belt straps is a desirable and important factor for the protection of the occupant If the safety belt is not well adjusted to the occupant, the belt will not be loaded at the moment when the vehicle stops, the strain will not necessarily start from the anchoring points but may start at the contact point between the belt and the body, as the occupant will gain speed in relation to the vehicle when the slack in the strap is taken up Then the force will rise very abruptly and the critical velocity for some part of the human body may be exceeded, causing injury near the point of contact The critical velocity for the strap may also be exceeded, causing strap rupture without energy absorption in the strap, and injuries to the occupant may not be caused by the strap but by the occupant's impacting the interior of the car Thus there is also a relative collision velocity between the occupant and the safety device, that velocity should be as close to zero as possible if the maximum protecting capacity of the device is to be used The injury-protecting capacity of shock-absorbing paddings to a certain extent will be their ability to reduce the relative impact deceleration between the front limitation of the compartment and those parts of the human body for which the critical velocity is low

The best protecting capacity of a safety belt will be achieved if the force on the body is primarily acting on bony structures of the body which, due to their stiffness, can distribute the force over large parts of the body But, when the major part of the body decelerates under the influence of the belt, some other parts will have a possibility of moving some distance before being decelerated by their anchorage or the limitation of the body, this means that, even if the skeletal structures of the torso are protected, dangerous internal injuries may arise But it also means that the whole weight of the body will not load the belt from the beginning As the deformation of the car, the belt and the body has two components an elastic one and a plastic one, and some parts of the body will move more or less freely during deceleration it will be very difficult to reconstruct an accident without a knowledge of the magnitude of these components and it will also be difficult to find a figure for the deceleration that is representative for the whole body

CAR BARRIER IMPACT TESTS

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The problem of vibrations in the vehicle would not be less during these conditions and therefore it was necessary to find a reliable method for the recording of the velocity-time pattern for different structures and ascertain what properties accelerometers and tensiometers should have in order to be usable under these conditions

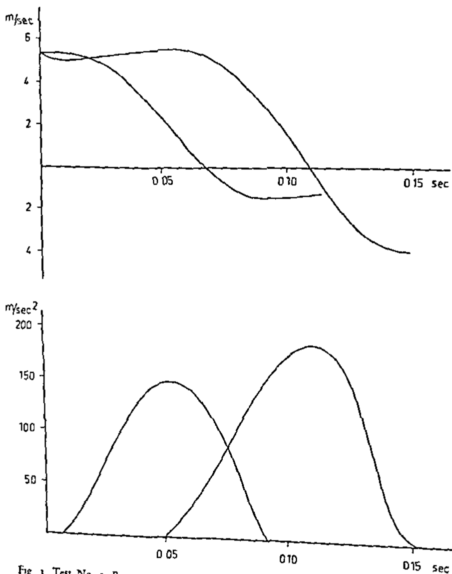


Fig 3 Test No 1 Restraint consisting of lap belt and upper torso restraint anchored to the floor

accelerated by means of gravity in a slope track In some tests it was possible also to use the engine of the car to gain speed before impact

The collisions were recorded by high-speed photography at frequencies of 1000—1500 frames per second, using a 16 mm high-speed camera

Results and Discussion

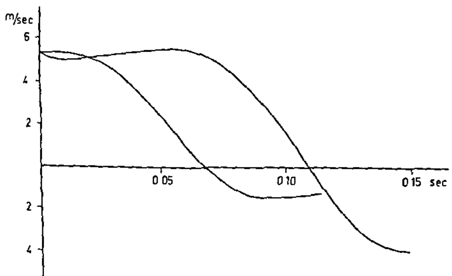
The car-barrier tests were carried out without much experience but at high cost and it was necessary to get as much information as possible from them A careful analysis of the high speed films was made with special attention to the deceleration pattern of the doorpost and the upper torso of the restrained dummy The curve-fitting method used was that of least squares, assuming a fifth degree polynomial for the velocity-time function In test No 2 the restraint broke very early, in test No 4 the restraint did not affect the upper torso in a desirable way and in test No 6 no restraint was used Figs 3—5 show the velocity-time plot and the deceleration-time plot for three of the tests using this method

In all these tests different kinds of long stretching body restraints were used and it appears from the deceleration curves that the dummies sustained about 30 per cent higher deceleration than the corresponding part of the cars but at least in some tests the rate of onset of deceleration was lower for the dummy Therefore it seemed of interest to make a more detailed study on a laboratory scale, where standardized conditions could be achieved

It was observed that in this speed interval between about 5—15 m/sec the average deceleration of European cars might vary between 15 and 30 g at a rate of onset of deceleration of 500—1500 g/sec and a duration of deceleration of about 100 msec However it was possible that for short periods of time, 20 msec or less, there might be peaks of much higher deceleration that would be smoothed out with the use of the least squares technique as that represents a lowering of the frequency response of the recording system The car-barrier impact would represent a more serious accident than a car to-car impact of equally heavy cars travelling at the same speed (HAYNES *et al* 1956)

In order to simulate a very serious situation for the restraint components it was decided to make laboratory tests with the various parts at an impact velocity of 10—15 m/sec an average deceleration of 100—150 g of the test vehicle a duration of 10 msec and rate of onset of deceleration of about 20 000—30 000 g/sec which would provide a reasonable safety margin for any car collision where the passenger compartment would remain sufficiently undamaged to protect the occupant from serious injuries

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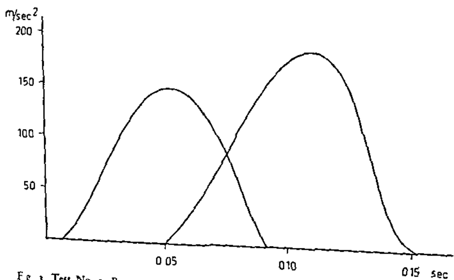


Fig 3 Test No 1 Restraint consisting of lap belt and upper torso restraint anchored to the floor

m/sec
12
10
8
6
4
2
0 05
0 10
0 15 sec
-2
-4

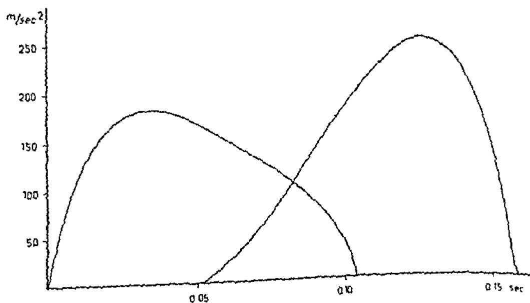


Fig 4 Test No 6 Single diagonal chest restraint anchored to the floor

m/sec
12
10
8
6
4
2
2
-4
0 05
0 10
0 15 sec

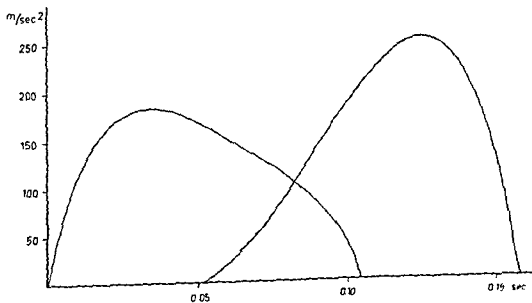


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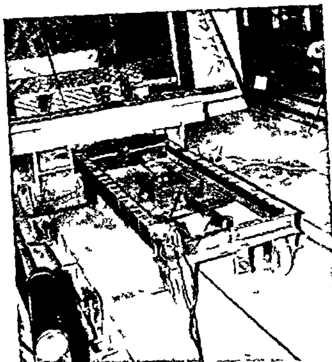


Fig 6 Test vehicle used for dynamic testing of experimental straps Strain gauge tensometers at each end of the straps were dynamically tested for later use in routine test with complete assemblies

These requirements led to the use of the following method which has been briefly described by ALDMAN & SIGMARK 1960

The test vehicle is accelerated to a velocity of about 9—12 m per second and is decelerated by the deformation of a lead cone in a distance of about 5—10 cm On the test vehicle is mounted a movable weight in form of a small vehicle restrained by a safety belt strap which restricts the motion of the weight in the direction of motion of the test vehicle On the test vehicle the strap and the weight are mounted small metal signals (Fig 6) These signals will reflect the light from a stroboscope lamp during deceleration These reflexes are photographed by a stereocamera and disturbing light is avoided by having everything but the signals covered with non reflecting black paint The stereocamera is so placed that the signals during deceleration will be in the field covered by both cameras The stroboscope used is a Philips PR 9100 which can give flashes of one microsecond s duration and of sufficient intensity at a frequency of about 300 flashes per second The frequency of

Against the background of the car-barrier tests it seemed of interest to investigate what accuracy could be achieved in the force-time pattern of dynamic tests of body restraints suitable for upper-torso deceleration. For this a basic requirement was to have a suitable number of observations during the impact period. The length of that period was to be determined in order to obtain the desired frequency of observations.

Though the data of the deceleration-time curve from many car collision tests could not be used for detailed information, the total length of the actual time interval could be calculated, as it would not be too much influenced by the above-mentioned errors and had often also been measured by other methods. It has been found to be about 100—250 msec and as a time-lag usually exists between the deceleration of the vehicle and that of the occupant even when he is restrained to the vehicle, the period of interest for this investigation would be 100—150 msec or less.

A frequency of observations of about 300 per second would then give 30—45 observations during impact time, which would be suitable if the measurements could be made with sufficient accuracy. The exactness of the recording methods is highly dependent on the possibility of measuring distances with a sufficient degree of accuracy at the desired frequency or the accuracy with which one point or signal can be recorded in space. The recording of the signal in three dimensions would mean a very great improvement compared with the high-speed film technique, which would be very difficult to evaluate in figures. A stereographic recording was therefore the first requirement for a better method.

Distortion of the recorded picture should be avoided as much as possible. The characteristics of the objective lens system should be tested. Consecutive recordings should be made by the same lens system. Deformation of the negative material between exposure and measuring should be minimized by using glass plates instead of film. Recordings should be made at sufficiently high frequencies, or about 300 cycles per second. The number of measurements should be kept as low as possible.

In the high speed film recordings the movement of a signal is measured as the difference in distance between the signal and a fixed object. The distance measurement contains the error of defining both the signal and the fixed object in consecutive frames. If the whole movement of the signal could be recorded in one stereopair it would mean that the distance measurement could be made between consecutive recordings of the signal in a three-dimensional coordinate system.

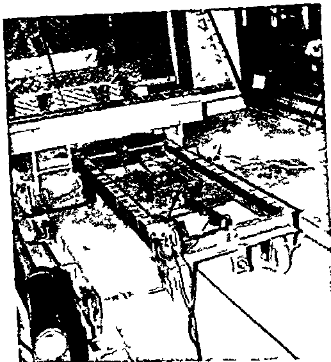


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On both plates the reflexes from the stroboscope flashes formed a series of points from each signal With the use of a Zeiss stereocomparator as a measuring instrument, the coordinates in space can be found and the distance that each signal has moved between two exposures can be calculated according to the Pythagorean formula The average velocity in each intervals is then determined according to the formula

$$v = \frac{\Delta s}{\Delta t}$$

For determination of the error in this formula tests have shown that disappearance of single flashes which occurs very seldom does not change the frequency to any measurable extent The dropped flash is usually no problem in measuring the plates, where it is easily identified as it will occur at different velocities in different signals The change in frequency of the stroboscope during a long period was less than 1 per cent During the short period that the collision takes place the frequency has then been considered constant

Thus the error in velocity orginates from the determination of Δs In the instrument used and at a photography distance of 2 8 m the standard error of Δs is about 0 5 mm Thus the standard error of the velocity is

$$M_v = \frac{v}{\Delta s} M_{\Delta s}$$

To check the geometrical conditions of the camera a straight line marked at 20 0 cm intervals was placed in the background Thus one of the most serious errors (φ-error or vergence error) can be checked To distinguish different signals a constant light of less intensity than the stroboscope flash can be used together with the stroboscope lamp The constant light will then form a thin line between all the stroboscope reflexes from each signal

Calculating the velocity according to the formula gives corresponding values of v and t The function is adjusted according to the method of least squares assuming a fifth-degree polynomial

$$v = a + bt + ct^2 + dt^3 + et^4 + ft^5$$

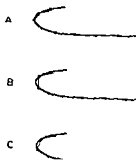


Fig 8 Three possible positions for signals close to zero velocity Since the distance in space between consecutive positions is determined A represents the case where the error is minimal B represents the case where the error is maximal and C any other case

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The camera used in these experiments was a Wild stereocamera with 40 cm base adjusted to take stereopairs according to the normal case The camera constant was 91 mm and the size of the pictures 6 5 x 9 cm The maximum aperture of the camera used was f 12 This rather small aperture made it necessary to use high speed plates Fig 7 shows the radial distortion curve of the stereocamera lens systems according to HALLERT's grid method (HALLERT 1954—55) The room was darkened and the camera shutter opened and after a last control of the stroboscope frequency when the intensity was increased the test vehicle was started When the collision was over and the stroboscope automatically switched off it was usually necessary to expose the frame marks separately, after which the shutter was closed

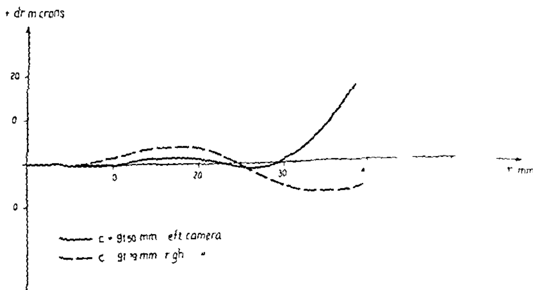


Fig 7 Radial distortion curve for the stereocamera used in the tests

On both plates the reflexes from the stroboscope flashes formed a series of points from each signal. With the use of a Zeiss stereocomparator as a measuring instrument, the coordinates in space can be found and the distance that each signal has moved between two exposures can be calculated according to the Pythagorean formula. The average velocity in each intervals is then determined according to the formula

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Thus the error in velocity originates from the determination of Δs. In the instrument used and at a photography distance of 2.8 m the standard error of Δs is about 0.5 mm. Thus the standard error of the velocity is

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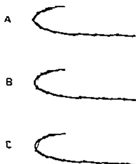


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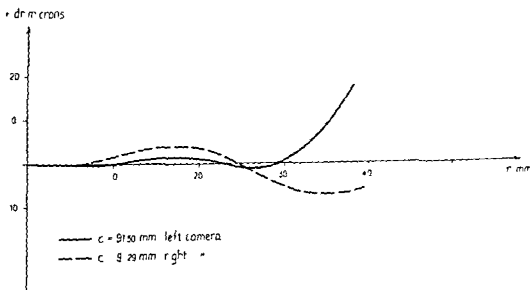


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Calculating the velocity according to the formula gives corresponding values of v and t The function is adjusted according to the method of least squares, assuming a fifth-degree polynomial

$$v = a + bt + ct^2 + dt^3 + et^4 + ft^5$$

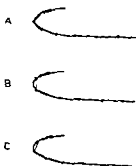


Fig 8 Three possible positions for signals close to zero velocity Since the distance in space between consecutive positions is determined A represents the case where the error is minimal B represents the case where the error is maximal and C any other case

When this function is known, the deceleration can easily be found by numerical derivation For known values of the deceleration the force can be calculated according to the Newton formula

The measuring of the elongation of the strap has a standard error of 0 5 mm

In calculation of the velocity there is a possibility under certain circumstances of another error in one interval of the curve That is when the velocity for one signal changes from positive to negative The magnitude of this error depends on the stroboscope frequency, on which part of the interval the change takes place, and on the arrangements of the experiment (Fig 8)

This error will appear only if zero velocity is passed, it appears only in one interval and it is reduced by smoothing the curve according to the method of least squares

During the first experiments only a third-degree polynomial for the velocity was used, but, as the standard error of unit weight for the velocity was calculated, it was found that a closer agreement between the standard error calculated theoretically from the accuracy in measurements and the standard error calculated from the record was better using a fifth-degree polynomial Polynomials of higher degrees did not give better agreement at the frequencies used in these experiments It may also be observed that a polynomial of the fifth degree gives, by derivation, the acceleration and hence the force as a polynomial of the fourth degree, which is necessary to give a satisfactory agreement with the direct force measurements, as will be described later on

If the standard error (in a function of adjusted quantities) in each point on the curve is calculated, errors in the comparator readings can easily be controlled With this method it was possible to determine the velocity, the deceleration and the force as a function of time but also the stress-strain curve and the stretching as a function of time could be plotted Even at this relatively low frequency of exposures it was evident that the stretching of the strap varied in different parts of the strap at the same moment

Results and Discussion

With the photogrammetric method described here, using an ordinary stereocamera with 40 cm base, it was possible to obtain velocity-time curves and stress-strain curves from different parts of the straps during dynamic stretching In Figs 9—12 the velocity as a function of time of different parts of the strap obtained with this method is shown for webbing containing polyester fibre The deceleration, the force and the stretching velocity as a function of time have also been calculated and are shown in these figures as are the corresponding stress-strain diagrams

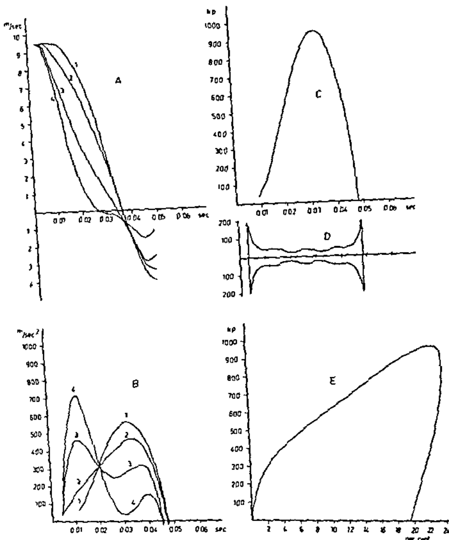


Fig 9 Results from dynamic testing of an experimental safety belt strap

A Velocity as a function of time for different parts of the strap, 1 = signal on the loading weight 2 – signal close to the weight, 3 = signal in the middle of the strap, 4 = signal close to the anchoring point in the test vehicle

B Deceleration as a function of time for corresponding signals. The rate of onset of deceleration is changed by the elongation of the webbing, peak deceleration is decreased

C The force as a function of time calculated from curve 1 in diagram B

D Standard error in the determination of the acting force

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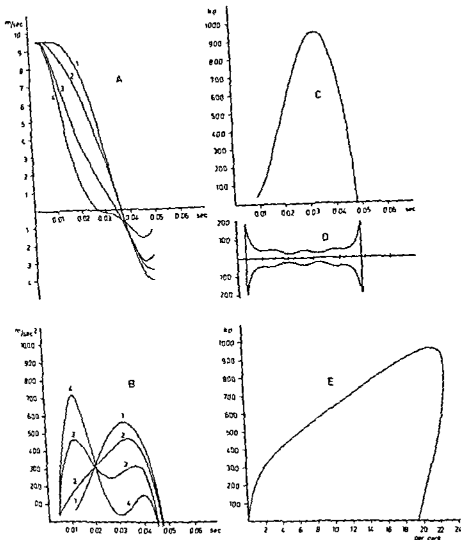


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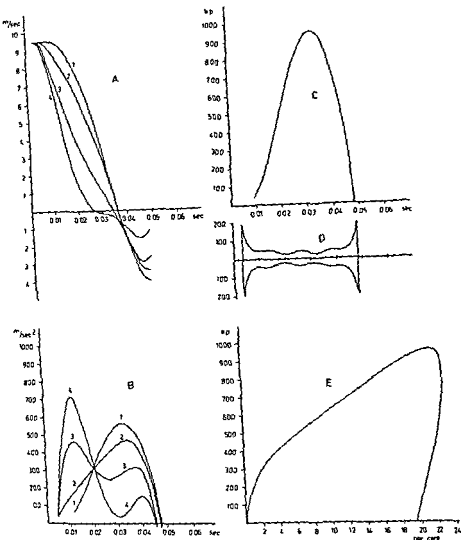


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C The force as a function of time calculated from curve 1 in diagram B

D Standard error in the determination of the acting force

E Dynamically obtained stress strain diagram. Standard error in elongation determinations is ± 0.1 % It should be noted that the rebound slope of this curve represents a mean from different parts of the strap The degree of elongation has been found to differ as much as 5 per cent between the two halves of the same strap

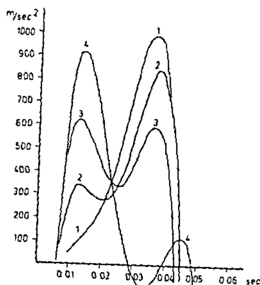


Fig 10 Deceleration-time patterns for different signals in a short stretching polyester strap demonstrating that the rate of onset of deceleration is changed by the elongation of the strap, and that the peak deceleration is not necessarily decreased thereby (the loading weight and the strap constitute a mass spring system)

The curves are numbered in the same order as in Fig 9

This example indicates the necessity for a standardized dynamic test procedure for body restraints, in which the input pulse is of short duration compared to the output pulse Load response patterns for different inputs may thus be calculated

Fig 11 Dynamically (1) and statistically (2) obtained stress strain diagrams of the same type of polyester webbing The different dynamic loadings were obtained by using weights of 16 and 31 kg at an impact speed of 9 5 m/sec and a weight of 26 kg at 12 m/sec Up to an impact speed of 12 m/sec straps of this type take up somewhat more energy (represented by the area under each curve) during dynamic loading than could be expected from statical testing, this effect being more pronounced at lower loads

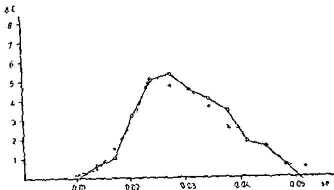


Fig 12 Stretching velocity as a function of time in a strap during dynamic testing The stretching velocity is expressed as the difference in elongation (in per cent of original length) between consecutive time intervals at a frequency of 290 stroboscope flashes per second The whole line combines the measured values for the foremost half of the strap and the dashed line the values for the rearmost half

It appears from these tests that there is a decrease in the rate of onset of the deceleration from the vehicle to the restrained weight and that this decrease is due to the elongation of the webbing

For some of the straps at least there was also a decrease in peak deceleration during these test conditions

In the stess-strain curves obtained dynamically with this method some of the straps take up more energy, represented by the area under each curve, than the same straps do when tested statically (Fig 11) In some curves the rebound is different in different parts of the strap, indicating that the stretching process is not the same along the whole strap

From the curve of the stretching velocity as a function of time also it can be seen that the stretching process is not identical for different parts of the strap This fact proves that the strain waves pass along the strap at a velocity low enough to be recorded with this method

With the frequency used, the time for one passage of the strain wave could be estimated at about 2—3 msec , and, as ' the wave" would probably contain different amplitudes travelling at various speeds interference phenomena could occur, causing stress concentrations in different parts of the strap (explaining the variation in the rebound of the stress-strain curve), so that the best opportunity to study the phenomenon would occur at the beginning of the stretching period.

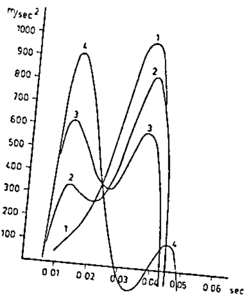


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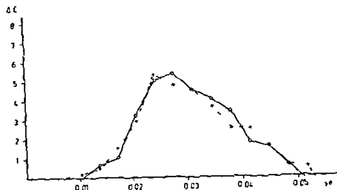


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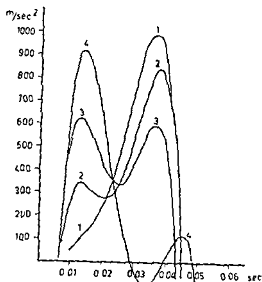


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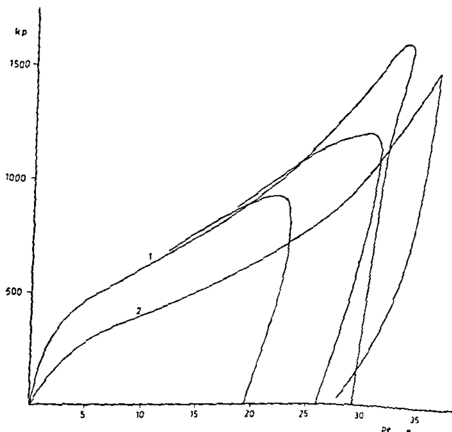


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The result of some tests with this method are shown in Fig 13 The film has moved in the direction of the y axis and the strap in the direction of the x axis A series of parallel dark oblique lines are seen starting from the hindmost part of the strap and reaching the foremost end of it in about one millisecond The distance between the end points is at the beginning 25 cm, giving a velocity of 250 m/sec for the stress-strain wave in this strap The

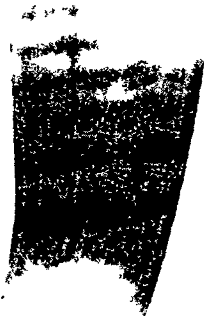


Fig 13 Strain waves passing the strap several times during elongation in dynamic testing The drum camera film has moved in a vertical plane parallel to the moving direction of the strap (which in the picture is from the left to the right) When the hindmost anchorage (to the left) stops a strain wave (the dark oblique line) starts reaching the foremost anchorage after one msec. Next strain wave starts two msec after the first one etc.

METHOD FOR RECORDING OF STRAIN WAVES

In the laboratory experiments the deceleration of the test vehicle is rather high, the rate of onset of its deceleration is very high and the vehicle is brought to rest within a fraction of the time used for stretching of the strap If the strap has no slack before impact, the force arising at the anchoring point of the strap in the test vehicle will create a strain wave which will start at that point and travel along the strap several times during elongation being reflected at the end points of the strap

The velocities of strain waves in the strap is of great interest If the test vehicle's velocity is high enough and the duration of the test vehicle's deceleration is short enough the strap will rupture even before the weight is decelerated, i e before the inertia of the weight produces any force to the strap at all In other words, if the strap is stretched faster than the material can elongate, the strap will rupture The lowest velocity for which this phenomenon can occur is known as the critical velocity, and this velocity can be estimated when the velocities of the strain waves are known

To study the velocity of the stress-strain wave with strain-gauge tensiometers at the anchoring points would be rather difficult in the test vehicle, as there will be stress-strain waves in the anchoring bolts also and a series of vibrations will occur in the vehicle during impact

For recording of the strain wave a photographic method would be more suitable As it was not possible to use a higher frequency with the available stroboscope it was necessary to use a constant light but then it was also necessary to use moving negative material

The first method to be tried was as follows A drum camera with a suitable objective lens for the photography distance and a suitable number of revolutions for this purpose was used to photograph the light reflected from the strap under certain conditions during impact In order to be able to record the localized elongation as it moves along the strap the intensity of the reflected light must be sensitive to the state of elongation in each part of the strap The strap was therefore painted with a reflective paint (Codit, produced by the Minnesota Mining and Manufacturing Company) which consisted of a suspension of small glass pearls Each pearl acts as a tiny mirror The reflected light from a certain area of the strap is therefore proportional to the number of pearls in that area When the strap is stretched the number of pearls will spread over a larger area and the intensity of the reflected light will therefore decrease If the light conditions are suitably arranged this localized decrease in intensity of the reflected light can be recorded with the drum camera

Results and Discussion

The result of some tests with this method are shown in Fig 13 The film has moved in the direction of the y axis and the strap in the direction of the x axis A series of parallel dark oblique lines are seen starting from the hindmost part of the strap and reaching the foremost end of it in about one millisecond The distance between the end points is at the beginning 25 cm, giving a velocity of 250 m/sec for the stress strain wave in this strap The

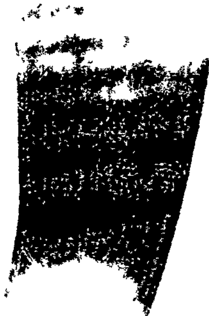


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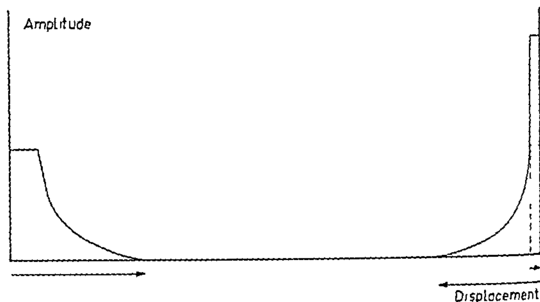


Fig. 14 The strain wave probably contains portions with different amplitude travelling at different velocity. The amplitude of the wave can therefore be increased already at the first reflex.

Philips PR 9100 stroboscope was used for timing. The reflected wave going from the foremost to the hindmost anchoring point cannot be seen on the picture but there is on the picture an interval of two milliseconds between the start of two consecutive waves, representing one millisecond for the passage in each direction. During the last part of the elongation the dark lines are closer together and this might indicate a different velocity of the wave. However, the wave might consist of several parts with different amplitudes travelling at different velocities, and it would then be unsafe to draw too many conclusions from that part of the stretching process using this method. From the curves in Fig. 12 it also appears that the stretching velocity is lower at the end of the elongation period.

Fig. 14 shows a theoretical example of the increase in force which may be the result of interference between waves of different velocity being reflected at the end points of a strap.

METHOD FOR RECORDING THE VELOCITY OF DEFORMING WAVES IN BONE STRUCTURES

With the methods described it is possible to record the deformation of the human body during impact. But in order to find one of the limiting factors for the deformation, i.e. the critical velocity of deformation it is desirable

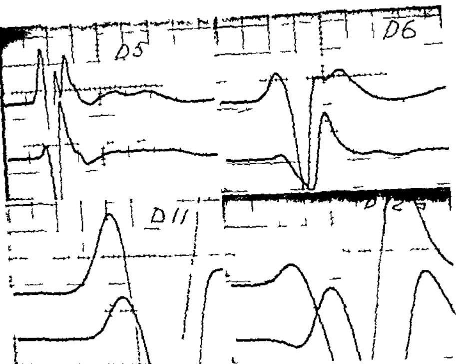


Fig 5 Oscilloscope recordings from these tests
Recordings D 5 and D 6 show the total responce of a single blow from the hammer In D and D 12 a he sweep velocity and the amplification have been increased to make time lag determinations easier All recordings where like in D 12 a the response started in opposite directions were discharded

In tests on adult objects the velocity of the deforming wave in bone structure was found to be 250—300 m/sec in lower leg and pelvis and 100—125 m/sec in the rib cage

to record the velocity of deforming waves in different skeletal parts of the body as those would act as anchorage for the restraints on the body

The test apparatus designed for these experiments consisted of two small piezo-electric accelerometers mounted on a stand so that they could be pressed against different parts of the body The impulses from these were fed to separate beams of a dual beam oscilloscope The time lag for the displacement of the accelerometers was recorded representing the time taken for the deformation wave to pass the distance between them Deformation was caused by a short blow from a hammer mounted as a pendulum near the site of one accelerometer Tests on the thorax were made at different degrees of air filling of the lungs and with open and closed glottis

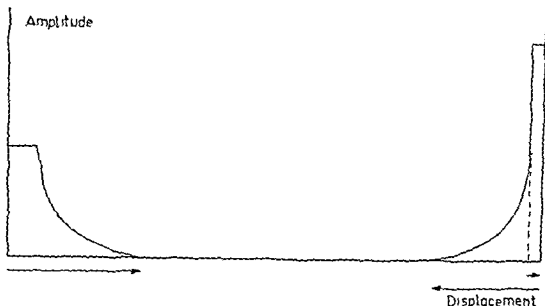


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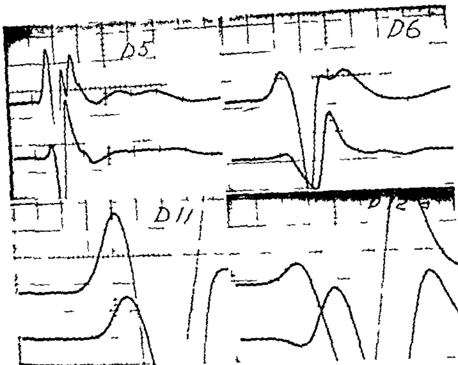


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Results and Discussion

Some results from these tests are shown in Fig 15 In these tests the weight of the hammer was kept as low as possible to get a well-defined response from the accelerometers The amplitude of the deforming waves was therefore small and it is possible that waves of different amplitudes may have different velocities, which might be of importance for the critical velocity *for deformation of the chest However, these figures* indicate that the combination of a low rigidity (Kulowski 1960) and a low velocity of the deforming waves of the chest must be taken into consideration when the rate of application of the force from an upper-torso restraint is predetermined This is still more important if the upper torso restraint has only a narrow contact surface on the chest

IMPROVED METHOD FOR RECORDING THE EFFECTS OF SHORT-TERM DECELERATIONS

In order to increase the accuracy of the recording method for dynamic loading of straps and studies on complete restraints, a special camera was built *This camera functions as a stereocamera but the image from* all the objective lenses falls on one large glass plate mounted on a rotating disc To distinguish it from other stereocameras this apparatus is called a disc stereocamera It can take ordinary stereo pictures according to the so called normal case in stereophotography but if the disc is rotated during exposure it is also *possible to record the movements of a series of signals illuminated* by a constant light

A special built-in timing device with a small flash lamp can then be used for timing up to a frequency of 1000 flashes per second with a variation of ± 1 per cent over longer periods Four objective lenses are used one pair is mounted with a base of 40 cm in the horizontal direction and the other with a base of 30 cm in the vertical direction Thus the rotation of the disc during exposure does not interfere with the possibility of determining the position of the signals at various intervals Every objective lens will record the movement of each signal as a curved line

In the stereocomparator specially built for measuring these plates it is possible to use the parallax readings from both of the combined stereocameras to identify the position of the signal on the curved lines in every position of the disc

It is possible to give the disc so high an angular velocity that displacement measurings can also be made in positions between the millisecond time marks, corresponding to frequencies higher tha

then a higher intensity of the illuminating light is of course required The frequency with which measurements can be made is dependent only on the limitations of the photographic recording system and the measuring instrument Theoretically it would be possible in this way to make continuous three-dimensional recordings of all objects where it is possible to use signals to represent the different parts of the objects, whether the object is moving or not

Results and Discussion

As the theories for measurements in recordings from this instrument are rather complicated and the camera has only recently been put into use in these tests, a more complete description of this technique will be published

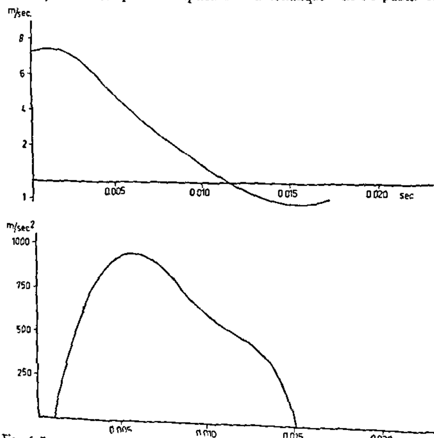


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Some results from these tests are shown in Fig 15 In these tests the weight of the hammer was kept as low as possible to get a well-defined response from the accelerometers The amplitude of the deforming waves was therefore small and it is possible that waves of different amplitudes may have different velocities, which might be of importance for the critical velocity for deformation of the chest However, these figures indicate that the combination of a low rigidity (Kulowski 1960) and a low velocity of the deforming waves of the chest must be taken into consideration when the rate of application of the force from an upper-torso restraint is predetermined This is still more important if the upper torso restraint has only a narrow contact surface on the chest

IMPROVED METHOD FOR RECORDING THE EFFECTS OF SHORT-TERM DECELERATIONS

In order to increase the accuracy of the recording method for dynamic loading of straps and studies on complete restraints, a special camera was built This camera functions as a stereocamera but the image from all the objective lenses falls on one large glass plate mounted on a rotating disc To distinguish it from other stereocameras this apparatus is called a disc stereocamera It can take ordinary stereo pictures according to the so called normal case in stereophotography but if the disc is rotated during exposure it is also possible to record the movements of a series of signals illuminated by a constant light

A special built-in timing device with a small flash lamp can then be used for timing up to a frequency of 1000 flashes per second with a variation of ± 1 per cent over longer periods Four objective lenses are used one pair is mounted with a base of 40 cm in the horizontal direction and the other with a base of 30 cm in the vertical direction Thus the rotation of the disc during exposure does not interfere with the possibility of determining the position of the signals at various intervals Every objective lens will record the movement of each signal as a curved line

In the stereocomparator specially built for measuring these plates it is possible to use the parallax readings from both of the combined stereocameras to identify the position of the signal on the curved lines in every position of the disc

It is possible to give the disc so high an angular velocity that displacement measurings can also be made in positions between the millisecond timing marks, corresponding to frequencies higher than 1000 flashes per second, but

separately Only the results from a test where a steel bullet impacts a lead plate is given in Fig 16 as a comparison with the tests of the accuracy in high-speed film analysis

The disc stereocamera is shown in Fig 17 and a strereocomparator specially disigned for this camera in Fig 18

THE STEERING WHEEL CATAPULT EFFECT

With the described methods it was possible to study how the force created by inertia at the deceleration of the vehicle is transmitted to the occupant by the restraint Now the question arose How will the fact that the legs and perhaps also the arms of the occupant are resting on certain parts of the vehicle influence the weight to be decelerated by the safety belt?

To help in answering this question the apparatus shown in Fig 19 was constructed It consists of a metal bar movable a certain distance in the horizontal direction By an elastic blow of a pendulum it is possible to accelerate the bar to a desired velocity within a short distance The stroke length of the bar and the pendulum is restricted by a lead plate and a nylon strap On the free end of the bar it is possible to mount a car steering wheel or a foot

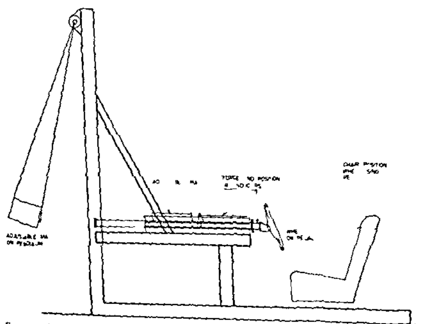


Fig 19 Steering wheel catapult.

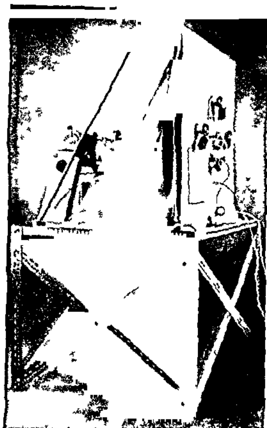


Fig 17 The disc stereocamera

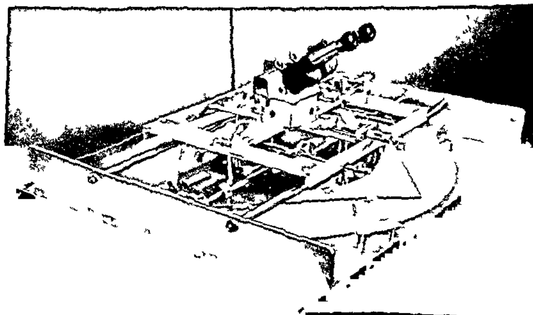


Fig 18 Stereocomparator designed for measuring the plates used in the disc stereocamera

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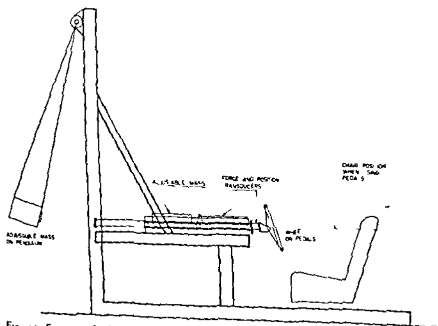


Fig 19 Steering wheel catapult.

rest The weight of the bar and the pendulum can be adjusted by additional weights The movement of the bar is recorded optically or electrically during each stroke The force used to decelerate the bar can then be determined In Figs 20—21 some recordings from these tests are shown

Results and Discussion

Like most problems in the impact acceleration field, this one is very complicated Test were made with a number of volunteers In some tests they could prepare for the impact by pressing down the steering wheel rim or the foot rest and in other tests they were sitting relaxed before impact The results

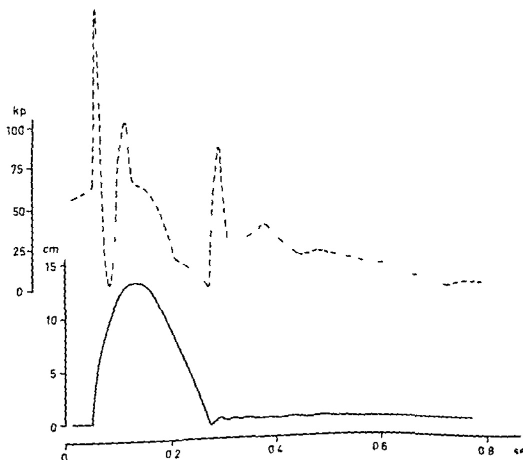


Fig 20 Force and displacement graphs from tests with the steering wheel catapult Dashed curve represents force and whole line curve displacement

The diagram is from a test where the object had prepared for the impact by pressing the steering wheel forwards with a force of about 50 kp Input speed 5 m/sec

varied considerably, and the differences were greater between tests with the same object than between the different objects

The average force used to stop the steering wheel before the end of the stroke varied from 15 to 65 kp, the higher values representing the situation where the object prepared for impact The total mass of the moving bar was 16 to 32 kg

Corresponding values for the foot rest was 35—106 kp, but here the position of the object seemed to interfere more with the results than it did in the steering-wheel series

An interesting observation in the steering-wheel series was that the spokes of the wheel acted like springs between the movable bar and the rim on which the hands rested In the recorded force time curves this caused a high force peak of short duration immediately followed by a short deflection in the curve before a steady plateau was reached This phenomenon was

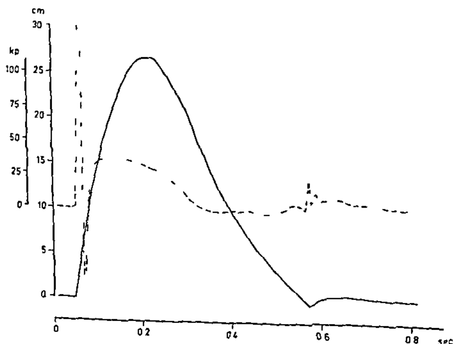


Fig 21 Force and displacement graphs from tests with the steering wheel catapult. Dashed curve represents force and whole line curve displacement
The diagram is from a test where the object sat relaxed before impact Impact speed was in both tests 5 m/sec

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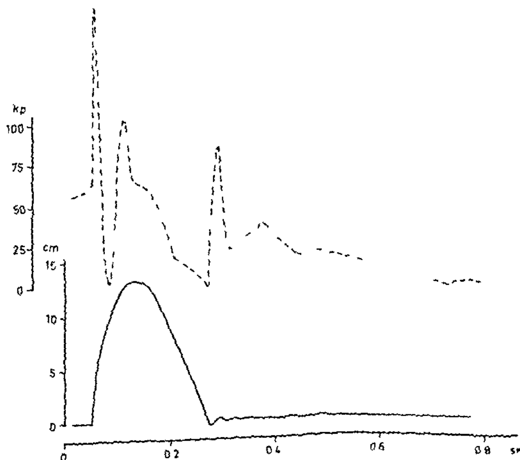


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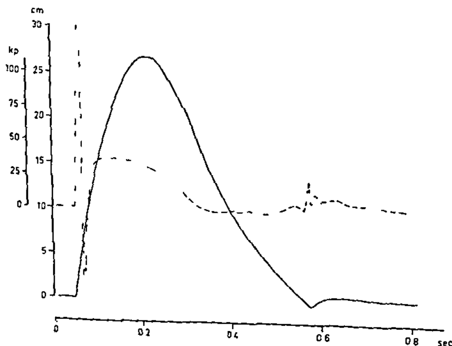


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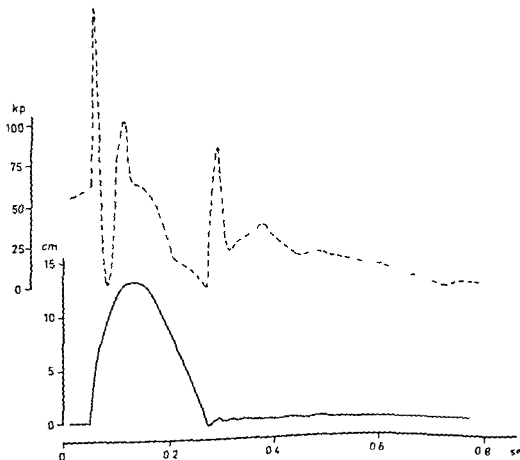


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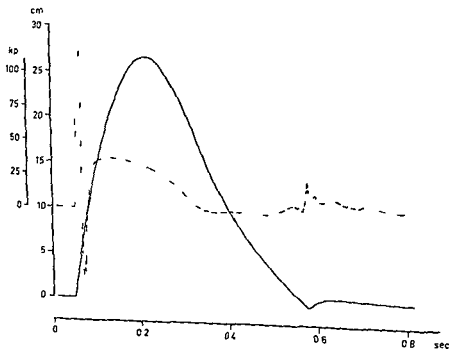


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obviously not merely an effect of the resistance in the object's arms, because a similar peak appeared when the steering-wheel rim was loaded by metal springs or rubber cords and a lower force peak was observed if the steering wheel rim was not loaded at all

This spring action of the spokes is obviously a desirable effect, as the velocity of the steering column during the first 5—10 msec was reduced by 2—3 m/sec, thus softening the shock on the arms in lowering the rate of application of the load The results, of course, varied considerably with different makes of steering wheels, but this effect deserves further observation particularly in connection with attempts to make collapsible steering-wheel columns

Another interesting observation from these tests was that after a few impacts the objects began to feel muscle soreness in the back of the neck It was quite obvious that the origin of this was a backward displacement of the shoulders at a very early moment of impact, which may contribute to a favourable orientation of the head before the $-G_x$ acceleration of the body begins

The part of the human body to be decelerated by contact with the vehicle is probably negligible at higher impact velocities, but at lower velocities and decelerations of about 10 G the driver's forearms and lower legs are probably not loading a long-stretching body restraint

STANDARDIZED DYNAMIC TESTING OF COMPLETE ASSEMBLIES

The behaviour of the human body during impact is influenced by the type of restraint used and by different positions of the anchoring points of each type of restraint The displacement of arms and legs will be of little importance as limiting factors, as their movements are not directly influenced by any actual type of restraint The movements of the torso and the head and the distribution of the pressure from the straps over the body may be of great importance To illustrate the action of different kind of restraints in this respect the high speed film can be used Figs 22—27 illustrate the movements of anthropometric dummies during a standardized impact test using different types of restraints





Fig 22 Restraint Type 1 single lap belt on living model and during dynamic testing 25, 50, 75 and 100 msec after impact

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Fig. 24. Restraint Type 3, lap belt with shoulder harness on living model and during dynamic testing 25, 50, 75, 100 and 150 msec. after impact





Fig 23 Restraint Type 2 single diagonal belt on living model and during dynamic testing 25 msec after impact If the strap is placed over the strongest part of the chest it will pass above the center of gravity During impact lower torso will then slide forward until it is stopped by the contact between the legs and the frontal limitation of the passenger compartment After impact the occupant is more or less free from the belt

Results and Discussion

In order to get comparable results from dynamic testing of different types of complete restraints the test procedure should be standardized as much as possible and should simulate the most severe conditions under which the restraint could be used Therefore the following tests were made The test vehicle was of a rigid construction that did not deform during impact The deceleration of the vehicle was determined by the deformation of a lead cone which cushioned the impact between the vehicle and the barrier On the vehicle was a bucket seat with tiltable backrest firmly secured, and holes were drilled in the steel members of the vehicle where the fittings of the restraints could be bolted in the correct positions The vehicle was accelerated by means of a falling weight to a speed of 40 ± 0 5 km/h which was reached



Fig. 24 Restraint Type 3 lap belt with shoulder harness on living model and during dynamic testing 25 50 75 100 and 150 msec after impact





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Fig 26 Restraint Type 5 combined lap and diagonal chest restraint with two anchoring points on the floor and one in the door *post, on living model* and during dynamic testing 25 50 75 100 and 125 msec after impact (cf Fig 27)








Fig. 25. Restraint Type 4: combined lap and diagonal chest restraint with all anchoring points on the floor: on living model and during dynamic testing 25, 50, 75, 100 and 125 msec after impact.



Fig 26 Restraint Type 5 combined lap and diagonal chest restraint with two anchoring points on the floor and one in the door post, on living model and during dynamic testing 25 50 75 100 and 125 msec after impact (cf Fig 27)



Fig 27 Restraint Type 5 with the dividing point between the lap strap and the diagonal chest strap in front of the hip on living model and during dynamic testing 25 msec after impact The lap strap was pulled away from the hip bone towards the abdomen and cut deeply into the soft abdominal part of the dummy at a very early moment during the deceleration (cf Fig 26)

1 metre before the barrier The stopping distance for the vehicle was 7 cm, providing a duration of the input pulse that was short compared with that of the restrained anthropometric dummy All restraints used were of the long-stretching type of webbing which had more than 25 per cent elongation at 1,500 kp static load

Five different types of restraints were used

Type 1 was a single lap belt anchored at two points in the floor one on each side of the seat (Fig 22)

Type 2 was a single diagonal belt anchored to the door post above the shoulder of the dummy and on the opposite side of the seat to the floor (Fig 23)

Type 3 was a lap belt with shoulder harness The lap belt anchored at one point to the floor behind the seat and attached to the lap belt in front

two shoulder straps anchored to the floor at another point behind the anchorage of the lap belt Fig 24 The shoulder harness was anchored to the floor at a distance from the seat corresponding to a point close to the rear seat in an ordinary European built car It should be noted that the force necessary to breake a rigid backrest is small compared to the force in the straps and that if the seat slides forward during impact the lap strap is usually pulled up towards the abdomen

Type 4 was a combined lap and diagonal chest restraint with all anchoring points in the floor on each side behind the seat Fig 25

Type 5 was a combined lap and diagonal chest restraint with one anchoring point on each side behind the seat for the lap strap and one anchoring point in the door post for the diagonal chest strap (Fig 26—27)

Only restraint Type 5 with the dividing point between the lap strap and the chest strap behind the hip could offer acceptable impact protection during these tests

METHOD FOR RECORDING THE DISPLACEMENT OF INTERNAL ORGANS

The movements of internal organs cannot be recorded by the methods described, but from the results it is obvious that when some types of restraints are used there is a possibility of a major displacement of such organs as the heart the liver etc Rupture of blood vessels in the neighbourhood of these organs is a well known type of injury in traffic accidents (KULOWSKI 1960) It would therefore be of great interest to record such displacement under different conditions and its effect on the blood vessels

To study the displacement of internal organs in $\pm G_x$ acceleration would for many reasons be very difficult and data on human tolerance for that situation are very limited (STAPP 1961) One problem to be overcome is that the body restraint will act as a mass-spring system and that the restraint may exert pressure on the abdomen, causing a pressure wave in the blood vessels that, at least at lower frequencies, would interfere with the results Another problem is that some organs will have a very complex movement, including rotation, in $\pm G_x$ accelerations of the body

For testing of a recording method and at the same time obtaining useful data, the $\pm G_z$ accelerations would be more suitable

For that purpose an angiographic roentgen technique would be useful, provided a sufficient degree of accuracy in displacement measuring could be reached (HERSHGOLD 1960)

An investigation was initiated with pigs as test animals The roentgen equipment could give sufficient exposure of the thoracic part of the animal during three milliseconds, using 120 kV and 320 mA A rollfilm changer was used that could allow single exposures in any part of the movement at a frequency of one exposure per second or serial exposures up to 6 exposures per second It was equipped with a photocell behind the film plane giving impulses at every exposure and a sighting aim for the tube with the following construction The centre of the screen was marked by a metal cross and above that, between the object and the tube, was an adjustable metal ring In the centre of that ring a thread holding a metal weight could be placed and the ring could be so adjusted that the centre of it was exactly vertically above the metal cross on the screen Then the weight was removed and a special fluorescent screen with a round central aperture was so placed that the metal cross was in the centre of the aperture The roentgen tube was then adjusted so that the shadow of the metal ring sight on irradiation fell symmetrically around the aperture The special fluorescent screen was removed and the test animal was fixed in a supine position to a fastening device on an oscillating table, which could be moved in the direction of the long axis of the animal The oscillating table could be moved with variable amplitude and frequency

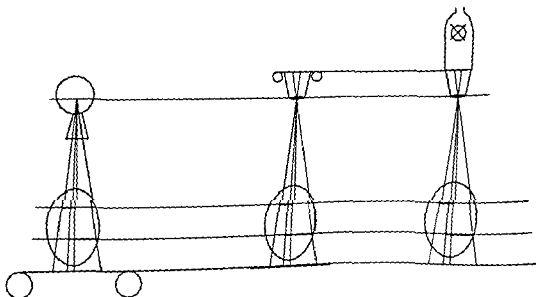


Fig 28 To the left the test animal in position between the film plane and the roentgen tube Two details in the body at different distance from the film plane are indicated To the right is shown how the roentgen film is photographed and the result [illegible] then projected through the same lens system on a table which can be elevated to enable measurements of the displacement of each detail at the distance they had from the focus of the roentgen tube thus eliminating the scale factor



Fig 29 The thoracic part of a test animal where contrast dye has been injected in the aorta When the heart is displaced in the cranial direction the left subclavian artery forms almost a 90 degree bend close to the aortic arch being stretched out again when the heart is displaced in caudal direction It should be noted that the angle between the left subclavian artery and the aorta is a common site of rupture of the aortic arch in car occupants killed in accidents Also the angle at which the intercostal arteries leave the aorta is considerably changed between the two positions indicating that the whole length of the aorta moves during the cycle

The deceleration at the different ends of the movement could to a certain extent be varied independently Urografin 65 % was used as a contrast medium, being injected through a polyethylene catheter in the inferior caval vein the right atrium or the aorta

Still reference exposures were first taken in different positions of the movement, after which the oscillating movement was started and serial exposures obtained at a frequency slightly different from that of the table in order to provide recordings from as many phases of the movement as possible

Reference lead signals in the body of the animal and on the table, together with the exposed ring-sight enabled reconstruction of real movements from the film pictures after the test (Fig 28) Thus the amplitude of the movements of the thoracic organs could be compared with the input pulse, i e the amplitude of the oscillating table at different frequencies

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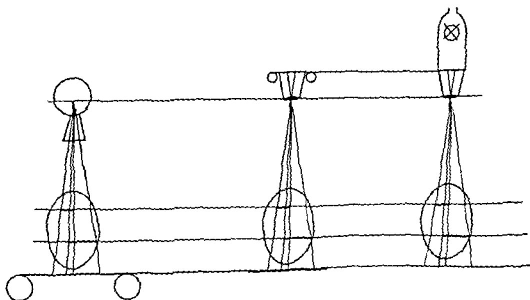


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Fig 31 Plastic mould of the heart and neighbouring vessels of a test animal Note the sharp bend of the subclavian artery around the clavicula probably limiting its downward displacement when the heart moves in the caudal direction Also note the angle at which the intercostal arteries leave the aorta (cf Fig 29)

As it has not yet been possible to take pictures in two planes synchronously, the movements were recorded first in a vertical and then in a horizontal plane. The disadvantage of not having synchronous exposures in both planes is partly offset by the great number of pictures in different phases of the movement in both planes separately

All animals were anaesthetized during the whole test procedure and were not allowed to regain consciousness before they were killed and autopsied some hours later The weight and position of different organs were registered and organs, vessels and bones were inspected for injuries The localization of the lead signals was also controlled.

The fact of its not yet being possible to have synchronous recording in two planes has made it desirable to restrict accelerations so as not to produce injury voluntarily, but to study varying degrees of displacement of different organs to variations in amplitude and frequency of the movement. The dis-





Fig 30 One test animal at autopsy had an haemorrhage in the wall of the left subclavian artery at its origin from the aortic arch Microscopic examination of the wall revealed a localized edema in the media close to the haemorrhage found between the media and the adventitia



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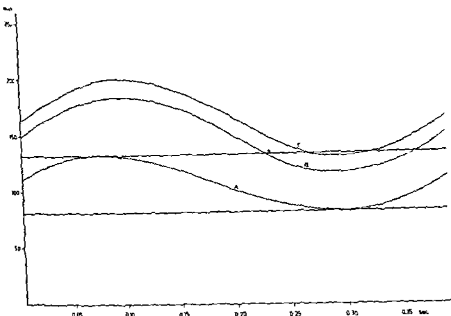


Fig 33 Displacement as a function of time for the position indicator on the oscillating table and two details on the heart at 2 5 cycles per second

Results and Discussion

The distance between the focus of the tube and the film plane was 100 cm After development, every film picture was photographed at a distance of 100 cm and with the main point adjusted according to the exposed metal ring sight to that of the roentgen exposure The negative was then projected through the objective lens used at exposure and the displacement measuring was made at the same distance from focus that the detail in question had during the roentgen exposure Care was taken that at projection also the main points coincided This could be checked by marking the position of different details at different projection distances All markings should then move radially from the main point at increasing distance (Fig 28)

To study the anatomy of the heart, plastic moulds were made of the heart and blood vessels of some test animals (Fig 31)

In Figs 32—34 displacement curves for different details in the thoracic part of one test animal are shown Though these curves are plotted from as many as 19 different exposures each in sinusoidal vibration at different frequencies, the accuracy of the distance measurements is no problem, but the time measurements for the different exposures in the interval must be

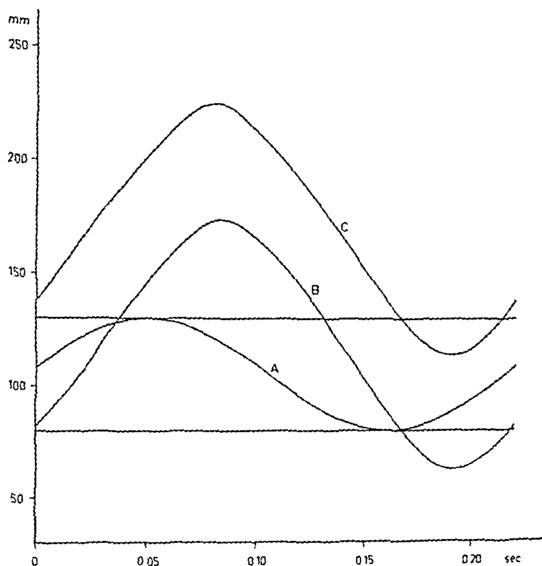


Fig. 32 Displacement as a function of time for the position indicator on the oscillating table and two details on the heart at 4.5 cycles per second

placement of the heart and neighbouring vessels during such a test is demonstrated in Figs 29—35

The impulses from the photocell on exposure and from a potentiometer on movements of the test table were fed to amplifiers with ink-jet galvanometers (Mingograph 24 B) and simultaneously recorded on paper. Thus the position and moving direction of the test animal could be determined at every exposure

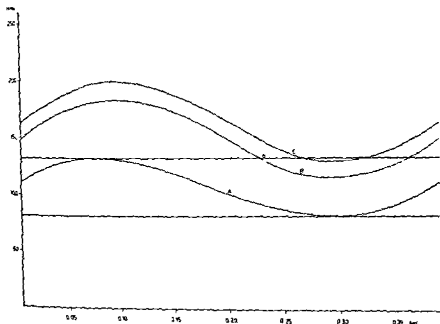


Fig 33 Displacement as a function of time for the position indicator on the oscillating table and two details on the heart at 2 5 cycles per second

Results and Discussion

The distance between the focus of the tube and the film plane was 100 cm After development, every film picture was photographed at a distance of 100 cm and with the main point adjusted according to the exposed metal ring sight to that of the roentgen exposure The negative was then projected through the objective lens used at exposure and the displacement measuring was made at the same distance from focus that the detail in question had during the roentgen exposure Care was taken that at projection also the main points coincided This could be checked by marking the position of different details at different projection distances All markings should then move radially from the main point at increasing distance (Fig 28)

To study the anatomy of the heart, plastic moulds were made of the heart and blood vessels of some test animals (Fig 31)

In Figs 32—34 displacement curves for different details in the thoracic part of one test animal are shown Though these curves are plotted from as many as 19 different exposures each in sinusoidal vibration at different frequencies, the accuracy of the distance measurements is no problem, but the time measurements for the different exposures in the interval must be

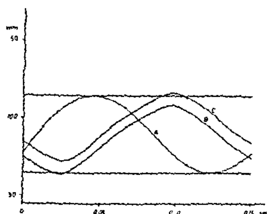


Fig 34 Displacement as a function of time for the position indicator on the oscillating table and two details on the heart at 67 cycles per second

made with an accuracy of about 5 msec, and here a position indicator on the oscillating table is of great help

Fig 35 shows the resonance curve for the heart of the test animal

If we let the heart represent a mass m in a mass-spring system with viscous damping where k is the spring constant and c the damping constant, and consider the steady-state response of this system to a harmonic force $F_o \cos \omega_l t$ then the equation of motion can be written

$$m\ddot{x} + c\dot{x} + kx = F_o \cos \omega_l t$$

The steady-state response of this system is (cf e g TONG 1959)

$$x = \frac{\delta_{st}}{\sqrt{\left(1-\frac{\omega_l^2}{\omega^2}\right)^2 + \left(2\frac{c}{c_c}\,\frac{\omega_l}{\omega}\right)^2}} \cos(\omega_l t - a)$$

where $\delta_{st} = \frac{F_o}{k}$ is the static deflection for the force F_o

$\omega = \sqrt{\frac{k}{m}}$ is the circular frequency of the undamped system

$c_c = 2\sqrt{mk}$ is the critical damping of the system

and $-a$ represents the phase lag

A graph of the magnification factor

$$a = \frac{1}{\sqrt{\left(1-\frac{\omega_l^2}{\omega^2}\right)^2 + \left(2\frac{c}{c_c}\,\frac{\omega_l}{\omega}\right)^2}}$$

as a function of the ratios

$\frac{\omega_l}{\omega}$ and $\frac{c}{c_c}$ can then be plotted

It can be shown that

a is maximum when $\frac{\omega_f}{\omega} = \frac{\omega_{fr}}{\omega} = \sqrt{1-2\left(\frac{c}{c_c}\right)^2}$

and that thus maximum is

$$a_{max} = \frac{1}{2\frac{c}{c_c}\sqrt{1-\left(\frac{c}{c_c}\right)^2}}$$

Then in the case shown in Fig 35

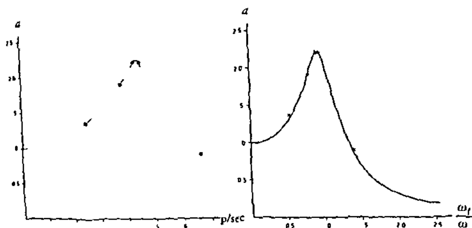


Fig 35 Magnification ratio as a function of frequency (dashed curve) and measured values compared to calculated curve of magnification ratio as a function of damping ratio and frequency ratio (whole line curve)

$a_{max} = 2\ 19$ for

$f = \frac{\omega_{fr}}{2\pi} = 4\ 73$ p/sec

Thus $\frac{c}{c_c}$ and $\frac{\omega_{fr}}{\omega}$ can be calculated

$\frac{c}{c_c} = 0\ 23$, $\frac{\omega_{fr}}{\omega} = 0\ 94$, hence $\omega = 31\ 6$ (sec)$^{-1}$

The two quantities $\frac{c}{c_c}$ and ω are of fundamental interest for analysing the response when the system is subjected to any type of mechanical action

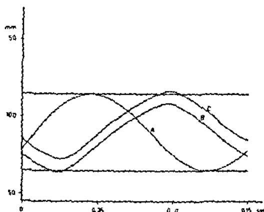


Fig 34 Displacement as a function of time for the position indicator on the oscillating table and two details on the heart at 6 7 cycles per second

made with an accuracy of about 5 msec, and here a position indicator on the oscillating table is of great help

Fig 35 shows the resonance curve for the heart of the test animal

If we let the heart represent a mass m in a mass-spring system with viscous damping where k is the spring constant and c the damping constant, and consider the steady-state response of this system to a harmonic force $F_0 \cos \omega_f t$ then the equation of motion can be written

$$mx + cx + kx = F_0 \cos \omega_f t$$

The steady-state response of this system is (cf e g TONG 1959)

$$x = \frac{\delta_{st}}{\sqrt{\left(1 - \frac{\omega_f^2}{\omega^2}\right)^2 + \left(2 \frac{c}{c_c} \frac{\omega_f}{\omega}\right)^2}} \cos (\omega_f t - \alpha)$$

where $\delta_{st} = \frac{F_0}{k}$ is the static deflection for the force F_0

$\omega = \sqrt{\frac{k}{m}}$ is the circular frequency of the undamped system

$c_c = 2\sqrt{mk}$ is the critical damping of the system

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A graph of the magnification factor

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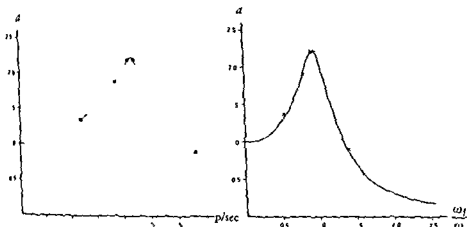


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The two quantities $\frac{c}{c_c}$ and ω are of fundamental interest for analysing the response when the system is subjected to any type of mechanical action

GENERAL DISCUSSION

BASIC PROBLEMS

On page 12 is described the problem whether, in ordinary car accidents the duration of the deceleration corresponds to the short duration impact zone in KORNHAUSER's sensitivity curve The problem cannot be considered to be solved, as enough information from experimental work in this field is lacking As body restraints are now being produced and used as crash-survival devices for ground vehicles there is an urgent need for research in the field of human tolerance As the situation now is, it is possible neither to decide in detail what properties a car safety belt should have in order to provide optimal protection in an accident nor to judge the reason for failure in certain accidents

A statistical analysis of accident material will therefore not answer the question to what extent it is possible to protect car occupants by a body restraint but only the question how effective existing safety belts are in this respect

The author has therefore chosen, in this work, to present some considerations concerning the necessity of studying the effect of certain factors on the restrained body, to present methods by which this effect can be studied and some conclusions which can be drawn from the limited results already available from such studies The experimental work along these lines is being continued and further results will be presented in later publications

DEFORMATION OF THE RESTRAINTS

In the introduction four questions to be answered in this publication were posed The first question whether it would be possible to record with sufficient accuracy the effect of peak decelerations with a duration of 20 msec or less, has been dealt with in the *dynamic testing of the straps*, where the input has a duration of that magnitude In the recording of strain waves in the strap and the velocity recordings of deformation of bone structure the

effect of such short term peak deceleration are studied and the question can therefore be considered to be answered in the affirmative

The second question about what deformation could be recorded in the restraint material under such peak decelerations has also been answered by the results of the dynamic testing of the straps The testing of the straps has resulted in dynamically obtained stress-strain diagrams showing that a greater *amount of energy is taken up in* the strap during stretching than could be expecte

impact

existen

amplitudes of these waves have given information about the complex nature of the elongation of the textile material used and demonstrated the need for dynamic testing of body restraints

DISPLACEMENT AND DEFORMATION OF THE RESTRAINED BODY

The third of the questions posed in the introduction has also been answered by this publication

The displacement of the body as a whole during the conditions prevailing in head-on car collisions can be studied by high-speed photography with the use either of dummies or of human volunteers Human-volunteer tests have not been included in this publication, though a limited number of such tests have been performed and to some extent information from real car accidents has been used for the planning of the actual tests Owing to the existing controversy about human tolerance or tolerances in this zone of impact acceleration it has been considered necessary to obtain more information about the possibility of predetermining the response of the restrained body to a given input pulse before human volunteers are used more extensively in such tests

In the roentgen recordings it has been possible to study the displacement of internal organs under the most simple conditions, i e in $\pm G_x$ accelerations with sufficiently high accuracy to obtain resonance curves for the heart of test animals in this direction During these tests it has been possible to demonstrate stress concentration on certain points of the blood vessels, indicating a risk for serious injuries to the human body in the corresponding situation However it is possible to use the same *method* for $\pm G_x$ acceleration also, although the technique might be somewhat more complicated, and such test are also being conducted with pigs as test animals

The disc stereocamera was originally designed for the study of the stretching process in the strap, but is now also used for recordings of the deformation

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of various parts of the human body The signals used as measuring points in these tests can be made with an extremely small mass and can be effectively anchored to the skin or to skeletal parts of the body of a test animal or a human volunteer so that it can be representative for displacement recordings of that part of the body

The fourth question in the introduction deals with the problem of what are the limiting factors for the effectiveness of a body restraint under conditions where high peak decelerations of very short duration act upon it This problem has not been completely solved by the experiments covered by this publication The need for basic data on human tolerances to mechanical forces in many fields for human activity has been considered so urgent that the author has restricted this publication mainly to the presentation of methods for such studies However, some aspects of the problem have been mentioned As the primary purpose of the body restraint is to prevent an impact with the interior of the vehicle, a rupture of the restraint at any time where the velocity of the restrained body has not been effectively reduced to prevent injury from such an impact would represent a failure to achieve that purpose The necessity of testing the restraint dynamically under such conditions that the critical velocity will not be reached in any car collision has been pointed out Thus the critical velocity of the restraint is one limiting factor for the efficiency of a body restraint

THE CAR SAFETY BELT

When designing a body restraint for ground vehicles many problems must be taken into consideration First of all such a restraint should be so cheap and so easy to use that it can be bought, installed and used in daily traffic WHITE (1960) has calculated the risk of lethal injury for a human body impacting a hard surface to be 50 % at a velocity of about 8 m/sec or less than 30 km/h But a body restraint used as a car safety belt should not only protect the occupant from lethal injuries but prevent all injuries from $-G_x$ acceleration at low speed and reduce the risk of serious injuries at higher speed (MOORE 1958) It is therefore necessary to design the safety belt for use on every type of trip, but if that is done the belt can never provide optimal body support at higher velocities Thus the problem in this case is more complicated than that of the load response of a mass spring system

If the restraint is to give maximal protection at normal speed it should restrict unintentional displacement of the wearer's body as much as is possible without interfering with the driving, in order to prevent impact with hard surfaces in the vehicle The response of the human body to the impact of

the vehicle should thus be governed by the properties of the restraint Therefore it is necessary that the body should remain in an upright seated position during deceleration That implies the requirement of an upper-torso restraint in combination with a lap restraint The best protection will then be gained if the impact load is acting primarily on certain bony structures of the body, because of their rigidity and their ability to distribute the force over large parts of the body

There is, however, a great difference in the structure of the pelvis and the chest The chest has a much lower rigidity and therefore, during the first moments of deceleration, the chest is deformed much more than the pelvis If this deformation takes place in a very short time, fracture may occur owing to the fact that the velocity difference between those parts of the chest which are far from the safety belt and those which are near the safety belt exceeds the critical velocity for the chest In the pelvis, on the other hand, such velocity differences are not likely to occur, because of the rigidity of the pelvis The need for long stretching straps is thus more pronounced for the chest than for the pelvis Theoretically a body restraint for ground vehicles could consist of a combination of straps consisting of short-stretching webbing in the lap restraint and long-stretching webbing, giving a lower rate of application in the upper torso restraint

But if the rate of application of the force has to be kept low on the upper torso it is also important that the upper-torso restraint should be well adjusted to the wearer Otherwise he would impact against the restraint and that would result in an unnecessarily high rate of application of the force

The arrangement of the straps must be such that even with such a moderate slack in the restraint as can be expected in public use, the load on the body will be distributed over the pelvic bone and the rib cage The diagonal strap should then not be attached to the lap strap in such a way that it can pull the lap strap away from the pelvic bone toward the abdomen The proper height of the upper anchoring point for the diagonal strap will therefore be dependent on the height of the seat, the distance between the seat and the side wall, etc The difference in body length will be of less importance for adults, since a tall occupant is usually heavier than a short one, the variation in the length of the torso is small compared to that of the legs, etc , so the position of the occupant's shoulder in relation to an anchoring point on the side wall will vary surprisingly little

SAFETY BELTS FOR CHILDREN

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not true of a child under that age It is a source of much controversy whether a child can be better protected with an ordinary safety belt in car accidents than without Statistics have shown that a child *runs less danger* than an adult in car accidents The skeletal parts of the body where the stress is concentrated by a restraint of this type, the pelvic bone and the rib cage, are comparatively smaller in a child, and this may indicate that a body restraint for children should be of a different construction in order to provide optimal stress distribution over the body Therefore not even a body restraint of the long-stretching type is to be recommended for children under the age of 7—10 years

THE DURATION OF THE DECELERATION

Thus with a long-stretching type of webbing for the straps it is possible to distribute the load over resistant body structures in a $-G_x$ acceleration in car accidents However, there remains the question How will the mass spring body system respond to such an acceleration?

As has been pointed out by STAPP and others, there is a time limit of 0 2 second for the appearance of fluid shifts in the body and with the space available in an automobile, that limit must not be exceeded if the occupants are to have optimal protection The corner duration in KORNHAUSER's load response curve is about 0 12 second and 0 125 second corresponds to one-half of four periods per second, which according to GOLDMAN and VON GIERKE (1960) would cause resonance at 800 G per second jolt for a peak of + 30 G_x in the sitting posture The same authors note that impedance measurements for the $-G_x$ acceleration are not available However, STAPP states that "The cardiovascular shock effect, characterized by pallor, sweating drop in blood pressure and rise in pulse rate, is related to rates of change of acceleration that correspond to dynamic loading rates for whole body resonance as shown by overshooting body acceleration peaks compared to those measured on the seat or vehicle Where the impact lasts longer than half a period of the resonant frequency transient cardiovascular shock signs have been observed in subjects sustaining 30 G peaks or higher of accelerative force applied either front-to-back or back-to-front Where force is applied for durations corresponding to less than half the period of resonant frequency resulting in higher dynamic responce, a lower applied peak G will incite higher over shooting peak G measured on the body, followed by rapid decline or rebound Mild transient cardiovascular reaction has been observed at less than 25 G peaks where duration was less than 0 1 second, and quite severe cardiovascular shock resulted from applications of 38 6 G at 1,370 G per second rate of

onset for 0 12 second total duration which corresponds to 8—9 cycles per second resonant frequency"

If possible, the duration should then be reduced to less than 0 12 second, even if that might imply some overshooting peak G on the body The limits for the duration will then be 0 04 and 0 12 second, and the closer to 0 12 second the duration comes the less will be the overshooting and the less will be the risk for damage to the rib cage

In the case of an automobile safety belt the situation is still more complicated As the lap restraint on impact will press the occupant down into the seat, which in most cars is soft, the acceleration vector will be a component of $-G_x$ and $-G_z$ where the G_z will depend upon the properties of the seat.

Even if the deformation of the webbing is mostly a plastic process there will be some elasticity and the human body also will be elastically deformed A rebound from the restraint is therefore most likely to occur The occupant will then rebound into the backrest of the seat, that rebound may be dangerous, but it can to some extent be controlled through a suitable design of the seat

During the forward displacement of the body it is an advantage if the body is supported by the seat So long as the seat does not place a dangerous additional load on the occupant a forward displacement of it to support the body is desirable Then the rebound of the body into the seat will be less dangerous if the seat is properly designed

A properly designed seat should slide forward under the guidance of an energy absorbing material so that no extra load is placed on the occupant, the forward motion should be limited to match the elongation of the body restraint and the upholstery should be designed to take care of the $-G_z$ acceleration and the $+ G_x$ acceleration on the rebound of the occupant

The proper design of the backrest of the seat must now be taken into consideration The problem whether an elevated backrest suitable for this situation would also protect the occupant from whiplash injuries in rear-end collisions is very complicated, because the posture of the occupant is probably of great importance in that connection

The risk of the driver s impacting the steering wheel can be eliminated by a collapsible steering column Studies on the steering-wheel catapult to find optimal limits for such a device are now going on

The problem of the rebound from the safety belt has not been included in this study for two reasons

(1) The problem is closely connected with the design of the seat, and the variations in seat design are so large that it has not yet been possible to standardize the tests

not true of a child under that age It is a source of much controversy whether a child can be better protected with an ordinary safety belt in car accidents than without Statistics have shown that a child runs less danger than an adult in car accidents The skeletal parts of the body where the stress is concentrated by a restraint of this type, the pelvic bone and the rib cage, are comparatively smaller in a child, and this may indicate that a body restraint for children should be of a different construction in order to provide optimal stress distribution over the body Therefore not even a body restraint of the long-stretching type is to be recommended for children under the age of 7—10 years

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also slides forward during impact LINDGREN (1961) has reported more than 200 accidents where a single diagonal has been used and where he has not been able to find any injuries which could be attributed to such a movement Nevertheless the risk exists Such cases have been reported by VON BAHR and ENGBERG (1961) and the present author has investigated some cases involving lethal injuries

At the same time it is evident that a properly designed safety belt can protect car occupants from serious injuries in accidents taking place at much higher speeds than was first supposed Therefore the Swedish Road Board, which is responsible for approval of car safety belts in this country, will in future only approve the most effective types for front seats

The effectiveness of a car safety belt is limited by the crashworthiness of the car, because it can only protect the occupant from impacting the interior of the vehicle as long as there is enough space available, and by the properties of the webbing, because the load on the upper torso must be distributed over an area greater than that under the strap but the elongation must be limited in order to prevent a secondary impact and to reduce the duration of the deceleration period To some extent the effectiveness of the belt is also limited by the design of the seat and of course by the fact that any load, from rear seat passenger or luggage, superimposed on the occupant during impact, may cause breakage of the restraint and the rib cage

Experience has shown that a proper safety belt can be effective in impacts in a sector of about 45° on both sides of the long axis of the car and even more for the occupant on the side opposite to the collision (SEVERY 1961)

(2) The problem seems to be of minor importance from a practical point of view, as pointed out below.

EXPERIENCE FROM PUBLIC USE OF SAFETY BELTS

Since the first national Swedish standards for safety belts were published in 1938, and the propaganda campaign for the use of belts started public acceptance has been very good. *In 1958 less than 10 % and in 1960 about 50 % of private cars were fitted with safety belts.* As the total number of private cars is a little more than one million and the total annual number of accidents reported by the police is about 60,000, with a total of about 10,000 injured or killed car occupants, *it should be possible to judge the effect of using a safety belt in traffic accidents.* There are, however, some difficulties about doing this. In the medical *statistics injuries sustained in traffic accidents* are not a present reported *in such a way that it is possible to compare this* material from the whole country *with the reports from the police. Some* tests are being made now *on the possibility to bring these two sets of material* together so that in a few *years it will be possible to get information from all* accidents about the injuries sustained *by all the occupants of the vehicles* involved.

During the period *1957—61 three main different types of safety belts* were in use. Of these the single diagonal belt is the most popular type, while the combined diagonal-lap belt restraint has come into more common use only during the last year. Therefore it has not yet been possible to get sufficient accident material concerning this type of restraint. The author has *therefore investigated only such accidents where the efficacy of the restraint* has been questioned and *as a complement* to these investigations has made an *inquiry among the officers in all police districts in the country about their* experience of *to what extent cars involved in accidents are equipped with* safety belts and to *what extent the belts in such cars are used in accidents.* The answers from about 400 districts with mainly rural traffic indicated that about two thirds of the officers were of the opinion that accident cars are *not very often equipped with safety belts and that when they are the belts are not very often used. If that is really the case it might explain why there* has not been a marked *decrease in the number of car occupants injured in* traffic accidents during the last two years.

During the whole period *only two or three cases have been reported where* a spinal injury in the *neck has been sustained by safety-belt users* and all these cases clearly *involved* both a very badly *adjusted restraint and a tiltable* seat backrest. The risk of *being injured by sliding from under the single* diagonal belt during *impact is greatly decreased by the fact that the seat*

also slides forward during impact LINDGREN (1961) has reported more than 200 accidents where a single diagonal has been used and where he has not been able to find any injuries which could be attributed to such a movement Nevertheless the risk exists Such cases have been reported by VON BAHR and ENGBERG (1961) and the present author has investigated some cases involving lethal injuries

At the same time it is evident that a properly designed safety belt can protect car occupants from serious injuries in accidents taking place at much higher speeds than was first supposed Therefore the Swedish Road Board, which is responsible for approval of car safety belts in this country, will in future only approve the most effective types for front seats

The effectiveness of a car safety belt is limited by the crashworthiness of the car, because it can only protect the occupant from impacting the interior of the vehicle as long as there is enough space available, and by the properties of the webbing, because the load on the upper torso must be distributed over an area greater than that under the strap but the elongation must be limited in order to prevent a secondary impact and to reduce the duration of the deceleration period To some extent the effectiveness of the belt is also limited by the design of the seat and of course by the fact that any load, from rear seat passenger or luggage superimposed on the occupant during impact, may cause breakage of the restraint and the rib cage

Experience has shown that a proper safety belt can be effective in impacts in a sector of about 45° on both sides of the long axis of the car and even more for the occupant on the side opposite to the collision (SEVERY 1961)

(2) The problem seems to be of minor importance from a practical point of view, as pointed out below

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Experience has shown that a proper safety belt can be effective in impacts in a sector of about 45° on both sides of the long axis of the car and even more for the occupant on the side opposite to the collision (SEVERY 1961)

SUMMARY

In the introduction the significance of the stretching properties of the straps in a body restraint is indicated and it is also pointed out that this problem has been very little discussed in the literature The difficulties involved in the study of effects of short-term, high peaks of deceleration are mentioned and the importance of such studies for the biodynamics of trauma is stressed

The review of earlier investigations includes the more important research activities in this field and some theories about the human tolerances to impact acceleration stress in the short-duration impact zone and that different opinions exist about the decisive physical quantity for injuries in this zone, indicating the need for more accurate recording methods

A critical analysis is made of the recording methods used in earlier work and the accuracy of high-speed photography is evaluated The background to and the planning of the present investigation are described in order to explain the procedure used in solving the problems

The duration of the deceleration period and the time lag between the *deceleration of vehicle and occupant, using different restraining devices in* European-built cars, were verified in car-barrier impact tests The experience from these tests showed the necessity of studying the elongation process in the straps under standardized laboratory conditions and also the need for better recording methods

The photogrammetric method for this study is presented, the accuracy and usefulness of this method is discussed and results from the study of dynamic testing of textile straps are presented

A method for recording strain waves in the straps is described

An equipment for the study of deforming waves in bone structures is described and some results from such studies reported

The disc stereocamera, a new application of photogrammetric principles for recording the deformation in various materials under dynamic load is briefly described and a test on the accuracy of this recording method is presented as a comparison with that of the high speed film technique

An apparatus called the steering-wheel catapult is described It was designed for a study of the extent to which the human body is decelerated by the

contact with the vehicle of arms and legs This catapult has also been found useful for investigating desirable qualities in the design of steering-wheel assemblies

High-speed film technique was used for the recording of the effect of different types of body restraints on anthropometric dummies under standardized conditions Results from these tests are given, illustrating the necessity of testing dynamically the effect of different strap arrangements in judging how effectively the load from the straps is distributed over structures suitable for taking up such high loads

A roentgen technique for displacement studies of internal organs during deceleration of the whole body is described, and its usefulness and accuracy are exemplified by the presentation of a resonance curve for the heart of a test animal, obtained by this method

In order to stimulate the collection of data, now lacking, on human tolerance to acceleration stress in the short-duration impact zone, this publication is mainly concerned with the presentation of methods for such studies, it reports a limited number of results only to prove the usefulness of the described methods The interrection between the safety belt and the seat of the car is discussed and the necessity of properly designed seats for optimal protection of car occupants in case of an accident is emphasized

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LIST OF SYMBOLS

Symbol	Meaning
a, b, c, d, e, f	constants in fifth-degree equation
a	magnification factor
c	damping constant in *mass-spring system*
c_c	critical damping
cm	centimeter
f	*frequency*
g	acceleration of gravity
G	unit to express inertial resultant to whole body acceleration *in multiples of* magnitude of the acceleration of gravity
$+G_x$	*transverse anterior posterior G, Eye Balls In*
$-G_x$	*transverse posterior-anterior G, Eye Balls Out*
$+G_z$	*positive G Eye Balls Down*
$-G_z$	*negative G, Eye Balls Up*
h	*hour*
k	spring constant
kp	kilopond, force unit correspondings to kg
kg	kilogram, weight unit corresponding to kp
km	kilometer
l	distance
m	meter
m	mass
mm	millimeter
msec	millisecond
M	standard error of unit weight
n	*number of observations*
p	*period*
s	*distance*
sec	second
t	time
t_f	duration of impact, or rise time of pulse
T_n	natural period of vibration
v	velocity
%	per cent
α	phase lag
δ	deflection in mass spring system
φ	vergence error in stereophotography
μ	standard error of velocity
ω	circular frequency
ζ_1	number of frame in unit weight system

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An experimental study in the cat

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Introduction

The somatotopically localized distribution of spinal impulses to the cerebellum has been well established by neurophysiological methods (SNIDER and STOWELL 1942 1944 ADRIAN 1943 and others) It has been found in experiments performed with pure tactile stimulation (SNIDER and STOWELL 1942, 1944) as well as in experiments where mixed exteroceptive and proprioceptive stimulation has been applied (ADRIAN 1943) However the anatomical organization of the pathways transmitting impulses from the spinal cord to the cerebellum is not sufficiently known to permit a complete correlation with the physiological data

Of the many routes transmitting spinal impulses to the cerebellum only the one via the lateral reticular nucleus appears to be anatomically organized in a somatotopical manner The spinal afferents to the nucleus terminate in a segmental pattern within it (BRODAL 1949), and the projection of the nucleus on to the anterior lobe of the cerebellum shows a fairly distinct localization (BRODAL 1943 see also JANSEN and BRODAL 1958) According to physiological observations this pathway appears to transmit impulses originating in cutaneous receptors (COMBS 1954 1956 BOHM 1953)

The chief route for impulses from proprioceptors to the cerebellum appears to be the dorsal and ventral spinocerebellar tracts (GRUNDFEST and CAMPBELL 1942 LUNDBERG and OSCARSSON 1956 OSCARSSON 1960) Anatomically these tracts however are generally considered to lack a somatotopical arrangement in their distribution within the cerebellum (MACNALTY and HORSLEY 1909 INGVAR 1918 JANSEN and BRODAL 1958) Evidence in favour of a somatotopical termination of spinocerebellar fibres has however been brought forward by CHANG and RUCH (1949) and more recently by YACHANANDA (1959)

The terminal areas for spinocerebellar fibres as studied by anatomical methods may be chiefly summarized as follows Fibres belonging to the dorsal spinocerebellar tract have been traced to the anterior lobe (BECK 1927 BRODAL and JANSEN 1941 ANDERSON 1943 WHITLOCK 1952 YOSS 1952 and several others) to Larsell s lobule VI (the same authors except for Anderson as well as others) lobule VIII (these authors Anderson and others) and lobule IX (the same authors as well

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as others). Some investigators have reported dorsal spinocerebellar fibres to Larsell's lobule VII (BECK 1927 and BRODAL and JANSEN 1941). Lobule X does not seem to receive such fibres (INGVAR 1918, BECK 1927, WHITLOCK 1952, YOSS 1952 and others), although spinocerebellar fibres have been observed within it in human material (BRODAL and JANSEN 1941). A few authors have traced dorsal spinocerebellar fibres to the paramedian lobule (BECK 1927 and ANDERSON 1943). Other cerebellar lobules do not seem to receive fibres from this tract, with the possible exception of the paraflocculus (INGVAR 1918). The cerebellar nuclei are generally regarded as not receiving fibres from it (BECK 1927, BRODAL and JANSEN 1941, WHITLOCK 1952 and others).

The anterior lobe seems to be the main site of termination for the dorsal spinocerebellar tract. Some investigators have reported an overweight of fibres to its anterior part, except for lobule I (INGVAR 1918, BECK 1927, BRODAL and JANSEN 1941 and VACHANANDA 1959). Lobules VI, VII, VIII and IX and the paramedian lobule are generally regarded as being less densely supplied with dorsal spinocerebellar fibres. Of these, however, lobule VIII seems to receive more fibres than the others.

Fibres belonging to the ventral spinocerebellar tract have been traced to the anterior lobe, lobules VI, VIII and IX (ANDERSON 1943, CHANG and RUCH 1949, YOSS 1953, VACHANANDA 1959). As compared with the dorsal tract, however, the ventral one seems to have a greater abundance of its fibres to the anterior lobe, and some investigators previous to those just cited have regarded the anterior lobe as the exclusive terminal cortical region for the tract (MACNALTY and HORSLEY 1909, INGVAR 1918 and BECK 1927).

In many of the investigations mentioned, attempts to determine separately the distribution of each of the two spinocerebellar tracts have been based upon studies of degenerating fibres in cases (human and experimental) where both tracts have been damaged simultaneously. The results of such investigations, however, cannot be regarded as decisive, since the tracts in part have common areas of termination. As regards the ventral spinocerebellar tract, there seems to be only four investigations of its intracerebellar distribution in which isolated transections of its fibres in the cord have been made (BRUCE 1910, CHANG and RUCH 1949, YOSS 1953, VACHANANDA 1959).

Another complicating factor in the differentiation between the particular terminal areas of the dorsal and ventral spinocerebellar tracts deserves mention. Some investigators (BRUCE 1910, YOSS 1953, MA

rion C SMITH 1957) have described a shift in the dorsal direction of ventral spinocerebellar fibres during their ascending course through the spinal cord It follows from this that a transection of the dorsal part of the lateral column generally assumed to harbour the dorsal spinocerebellar tract only may involve ventral spinocerebellar fibres as well

The aim of the present study is to attempt a detailed mapping of the terminal regions within the cerebellum of the two spinocerebellar tracts with particular reference to the question whether they terminate in a somatotopical manner or not Silver impregnation methods were used for this study since it may be expected that more clearcut results might be obtained in this way than by using the Marchi method. Silver impregnation methods will demonstrate not only myelinated fibres but unmyelinated as well if they are present Furthermore the degenerating terminal ramifications of the mossy fibres in the granular layer of the cerebellum can be identified and thus make possible an exact determination of the terminal areas

For the separate determination of the terminal regions of the two spinocerebellar tracts the dorsal shift of the ventral spinocerebellar fibres mentioned above represents a possible source of error In order to avoid this it was deemed necessary as a preliminary step to investigate in some detail the topography of the degenerating ascending fibres within the cross sectional area of the cord at various levels following lesions of the cord at caudal levels The results of these studies represent the first part of the present investigation

as others) Some investigators have reported dorsal spinocerebellar fibres to Larsell's *lobule VII* (BECK 1927 and BRODAL and JANSEN 1941) Lobule X does not seem to *receive such fibres* (INGVAR 1918 BECK 1927 WHITLOCK 1952 YOSS 1952 and others) although spinocerebellar fibres have been observed within it in human material (BRODAL and JANSEN 1941) A few authors have traced dorsal spinocerebellar *fibres to the paramedian lobule* (BECK 1927 and ANDERSON 1943) Other cerebellar lobules *do not seem to receive fibres from this* tract with the possible exception of the paraflocculus (INGVAR 1918) The cerebellar nuclei are *generally regarded as not receiving fibres* from it (BECK 1927 BRODAL and JANSEN 1941 WHITLOCK 1952 and *others*)

The anterior lobe seems to be the main *site of termination for* the dorsal spinocerebellar tract Some investigators have reported an overweight of fibres to its anterior part except for lobule I (INGVAR 1918 BECK 1927 BRODAL and JANSEN 1941 and VACHANANDA 1959) *Lobules VI VII VIII and IX and the paramedian lobule are generally* regarded as being less densely supplied with dorsal spinocerebellar fibres Of these however lobule VIII seems to receive more fibres than the others

Fibres belonging to the ventral spinocerebellar tract have been traced *to the anterior lobe lobules VI VIII and IX* (ANDERSON 1943 CHANG and RUCH 1949 YOSS 1953 VACHANANDA 1959) As compared with the dorsal tract however the ventral one seems to have a greater abundance of its fibres to the anterior lobe and some investigators previous to those *just cited have regarded the anterior lobe as the exclusive* terminal cortical region for the tract (MACNALTY and HORSLEY 1909 INGVAR 1918 and BECK 1927)

In many of the investigations mentioned attempts to determine *separately the distribution of each of the two spinocerebellar tracts* have been based *upon studies of degenerating fibres* in cases (human and experimental) where both tracts have been damaged simultaneously The results of such investigations however cannot be regarded as decisive since the tracts in part have common areas of termination *As regards the ventral spinocerebellar tract* there seems to be only four investigations of its intracerebellar distribution in which isolated transections of its fibres in the cord have been made (BRUCE 1910 CHANG and RUCH 1949 YOSS 1953 VACHANANDA 1959)

Another complicating factor in the *differentiation* between the particular terminal areas of the dorsal and ventral spinocerebellar tracts deserves mention Some investigators (BRUCE 1910 YOSS 1953 MA

mounted and stained according to van Gieson's method. Blocks taken from segments of the cord rostral to the lesion were treated according to SWANK and DAVENPORT's modified Marchi method (1935 a). Most of them were embedded in paraffin and cut in transverse sections at 30 μ, but frozen or celloidin sections cut at 20 μ were prepared from some blocks. Sections from almost every block were counterstained with thionine in order to facilitate distinction between the white and grey matter.

The cerebellum usually, after having been separated from the brain stem, was divided in the median plane and each half further subdivided by two sagittal sections, one just medial, the other just lateral to the paramedian lobule, thus giving three blocks from each half. Sagittal frozen sections were cut at 20 μ and collected in groups of fifteen. From each group one or more sections were selected for impregnation with silver according to the method of NAUTA (1957) in which the Laidlaw solution is used. In some cases the method of NAUTA and GYGAX (1954) was used instead or in addition. In many of the cases two or more additional sections from groups in which degenerating fibres were found in Nauta preparations were impregnated according to other silver methods (GLEES 1946, BEUMONT and LHERMITTE in Romeis 1948). In some cases folia from the anterior lobe were dissected free from the cerebella and cut in transverse sections. In the control cases sections were taken from all groups and impregnated according to the two Nauta methods as well as to the other two silver methods mentioned above. In some cases the dorsal and ventral paraflocculus together with the flocculus were separated from the rest of the cerebellum and cut serially. In these, however, the medialmost part of the dorsal paraflocculus was not included in the separate block but was left in the block containing the paramedian lobule. Drawings of selected sections from the spinal cord and the cerebellum were made by means of a projection apparatus.

In all cases with lesions in the upper cervical and mid lumbar regions the lesion was checked in relation to the caudal extent of the lateral cervical nucleus and the column of Clarke respectively.

Material and Methods

Many of the animals employed in the present investigation have also been used for a study of the morphology and temporal course of degeneration in cerebellar mossy fibres following transection of spino-cerebellar tracts (BRODAL and GRANT 1962) The experimental material for the present study consists of cerebella and spinal cords of 39 adult cats (selected from a total material of 69 animals) with lesions at various levels of the spinal cord and involving different parts of its cross sectional area Twenty-two cases were used for studies of the course of the ascending fibres in the spinal cord, 4 cases for studying the cerebellar projection area of the spinocerebellar tracts together, 13 cases for that of the dorsal and 13 cases for that of the ventral spinocerebellar tract Some cases used for the study of the fibre course in the spinal cord have been used in addition for the study of the cerebellar projection of the spinocerebellar tracts The cerebella of 3 animals removed in the same manner as the experimental ones served as normal controls

The experimental animals were anaesthetized with Nembutal (ip) and operated on with aseptic precautions Following laminectomy, usually extending over two neighbouring vertebrae the dura and arachnoid were split and part of the cord was exposed This step was performed with greatest care, special attention being paid to avoid pressure against and rotation of the spinal cord Saline was used to avoid drying of the nervous tissue An incision was made in the spinal cord Sometimes dorsal spinal root fibres were cut The dura was left unsutured Some animals were given penicillin (im) after the operation

After 4 to 73 days the animals were killed under Nembutal anaesthesia by intracardiac injection of saline acacia followed by formol saline acacia according to the method of KOENIG, GROAT and WINDLE (1945) The central nervous system, from some segments caudal to the lesion, up to the mesencephalon, was then dissected free and immersed in neutral formol saline solution (5 %) for at least three days

The part of the cord containing the lesion was then removed embedded in paraffin and cut serially in transverse sections at 10 μ Of each group of five successive sections the first section was mounted and stained with thionine and in several cases the second section was

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Chapter I

Spinal course of ventral and dorsal spinocerebellar tracts

In the following description the terms "degenerating fibres" and "degeneration" will be used synonymously. The ventral columns will be regarded as being delimited from the lateral ones by radial planes through the central canal and the points of exit of the most lateral of the ventral root fibres at the periphery of the cord. The lateral columns will be arbitrarily divided by a coronal plane passing through the central canal *into a ventro- and a dorsolateral part, which will* here be referred to as the ventrolateral and dorsolateral *quadrants*, respectively.

A detailed description of degeneration will be restricted to that present *in the lateral columns. The degeneration in the posterior columns* will not be described here. There is no case in this material with a lesion restricted to the ventral columns. Therefore it is not possible to determine in a decisive manner, whether these contain fibres turning into the ventrolateral quadrant of the spinal cord in their ascending course, and whether they contain fibres passing to the cerebellum. The degeneration in the ventral columns will therefore be only briefly commented upon.

Results

In the following presentation the material will be subdivided into three groups according to the degree of involvement of the two quadrants of the cross sectional area of the cord defined above.

A. Ventrolateral quadrant lesions
B. Ventrolateral quadrant lesions encroaching on the dorsolateral quadrant
C. Dorsolateral quadrant lesions

A consistent finding made in all cases is worth mentioning: a considerable number of the fibres in the three components of ascending degeneration (to be described below) are of a large ca-

1 Ventrolateral quadrant lesions

This type of lesion involved the ventrolateral quadrant with practically no involvement of the dorsolateral one Such lesions occur in 6 cats (*K 65, 76, 78, 80, 85 and 93* with survival periods of 5 days and with lesions between the 1st and 7th lumbar segments) All these cases demonstrate that there is a dorsal shift of the degenerating fibres as they ascend in the cord until they return in the upper cervical segments to a ventral position The findings are essentially the same in all six cases and are described in detail in the case of cat K 78

Cat K 78 (survival period 5 days) The lesion (Fig 1 A) at the level of the 1st lumbar segment on the left side comprises a ventral part of the ventrolateral quadrant and a lateral portion of the ventral column It does not encroach upon the grey matter

In the *12th thoracic* segment on the side of the lesion there is a rather broad band of degeneration at the periphery of the cord extending from the most lateral part of the ventral column to the dorsal part of the ventrolateral quadrant (Fig 1 B) The medial part of this band extends further dorsally than the peripheral one almost exactly to a plane passing through the central canal Some scattered degeneration is visible medial to the band as well as in the ventral column and the dorsolateral quadrant

In the *8th and 6th thoracic* segments the band of degeneration has a more peripheral location and is found more dorsally extending into the ventral part of the dorsolateral quadrant (Fig 1 C) It has about the shape of a comma with its thickest part dorsally where a narrow peripheral zone is free from degeneration There is some scattered degeneration in the ventral column and in the dorsolateral quadrant

In the *8th 6th and 4th cervical* segments there is a rather loosely arranged band of degeneration along the periphery of the ventrolateral quadrant extending into the ventral column and a more concentrated one peripherally in the ventral part of the dorsolateral quadrant (Fig 1 D) The latter contingent of degeneration extends dorsally to about the level of the cervix of the dorsal horn A few granules of degeneration are located rather peripherally in the dorsal part of the dorsolateral quadrant Some scattered degeneration is found in the central part of the dorsolateral quadrant and some in the ventral column

In the *2nd cervical* segment (Fig 1 E) the main mass of the degeneration is located more ventrally than in the lower cervical region namely peripherally in the dorsalmost part of the ventrolateral quadrant However there are still some degenerated fibres ventrally in the dorsolateral quadrant Some degeneration is found just lateral to the root entry zone as well as in the ventral column

As will be seen from the photograph of Fig 1 there are some degenerating fibres also on the side contralateral to the lesion

Essentially the same distribution of ascending degeneration is seen following hemisection of the cord at a level below the origin of the column of Clarke (I 3-4 BRYRD 1954) and this case is illustrated in detail

In *cat K 95* (survival period 73 days) the thionine sections (*Fig 2 A*) *show that* there is a subtotal hemisection of the 1st sacral segment Part of the ventral column medially and a superficial portion of the ventrolateral quadrant have been spared

In segments rostral to the lesion (*Figs 2 B* and *2 C*) except the first cervical there are two separate contingents of degenerating fibres both situated peripherally one at the dorsal extremity of the lateral column one more ventrally The dorsal contingent is restricted to the region just lateral to the root entry zone In the 1st cervical segment however this contingent is almost absent On close study degenerating fibres can be detected around the cells of the lateral cervical nucleus The ventral contingent of peripheral degeneration in segments rostral to the lesion seems to extend somewhat more dorsally than the corresponding contingent in cat K 78 (*cp Fig 2 C with Figs 1 C and D*)

In addition to confirming the course of the ascending fibres in the ventrolateral quadrant, this case (Fig 2) demonstrates that fibres coming from levels below the caudal *end of the column of Clarke* ascend in the dorsolateral quadrant to upper cervical levels A fairly massive contingent of these appears to end in the lateral cervical nucleus This *contingent* will be considered again, in connection with the analysis of ascending degeneration in the dorsolateral quadrant First, however, some other cases will be described

B Ventrolateral quadrant lesions encroaching on the dorsolateral quadrant

This type of lesion occurs in two cases (*cats K 79 and 92* with survival periods of 5 and 6 days, respectively and with lesions at the level of the 3rd and 1st lumbar segments respectively) In both cases the findings are essentially similar and are described in detail in the case of cat K 92

Cat K 92 (survival period 6 days) A ventrolateral incision was made in the 1st lumbar segment on the left side The lesion (Fig 3 A) comprises the entire ventrolateral quadrant and encroaches slightly upon the ventral portion of the dorsolateral quadrant especially at its periphery and also upon the lateral part of the *ventral horn*

In the *13th thoracic* segment (Fig 3 B) degeneration on the left side is found mainly peripherally within the field corresponding to the lesion

Within the dorsolateral quadrant *the degeneration is restricted to the ventral* portion whereas the dorsal part is entirely *free*

In the *10th and 7th thoracic* segments the degeneration has moved dorsally Its dorsal limit is found considerably *dorsal to the level of the caput of the dorsal horn* There is however no degeneration in the field just lateral to root entry zone

In the *5th and 3rd thoracic* segments (Fig 3 C) there is approximately the same dorsal extension of the degeneration but there seems to be a differentiation within the peripheral band of degeneration *for there is a* closer aggregation of degen

erating fibres about the level of the lateral horn These fibres correspond in position to the dorsal portion of the degeneration in the cases with ventrolateral lesions described above

In the *1st thoracic and 8th cervical* segments degeneration extends dorsally to the root entry zone (Fig 3 D) A closer aggregation of degenerating fibres is visible at the periphery of the cord extending dorsally to about the level of the cervix of the dorsal horn

In the *3rd cervical* segment (Fig 3 E) degeneration is found in the field just lateral to the root entry zone In addition the zone of heavy degeneration has shifted in a ventral direction

In the *1st cervical* segment (Fig 3 F) there are two areas peripherally with rather dense aggregation of degeneration The ventral one extends dorsally to about the level of the central canal The dorsal one is found at the periphery of the dorso lateral quadrant and leaves the peripheral region just lateral to the dorsal horn relatively free from degeneration The limit between the two contingents seems to coincide with the invagination of the surface of the lateral column (arrow in Fig 3 F)

In the case of cat K 79 the lesion as seen in thionine sections comprises the ventral part of the ventrolateral quadrant and encroaches upon the lateral part of the ventral column The Swank–Davenport preparations just above the lesion demonstrate however some degenerating fibres in the most ventral portion of the dorsolateral quadrant The course of the ipsilateral degenerating fibres corresponds to that in cat K 92 (Fig 3), and demonstrates that not only fibres ascending in the ventrolateral quadrant but also fibres ascending in the most ventral part of the dorsolateral quadrant undergo a dorsal shift during their course In cat K 79 there are also some degenerating fibres ascending contralaterally Since this case provides additional information concerning the course of fibres ascending in the dorsolateral quadrant it will be considered again below

C Dorsolateral quadrant lesions

In a total of 6 cats the lesions turned out to involve more or less of the dorsolateral quadrant without encroachment on the ventrolateral quadrant

In *cats K 59 61 68 71, 75 77* (survival periods 5 days) having lesions restricted to the dorsolateral quadrants in the upper lumbar (K 59 71 75) at the transition between the lumbar and the thoracic (K 77) and in the lower cervical (K 61, 68 Figs 4 A and 11 A) segments degeneration is seen at the periphery of the dorsolateral quadrant (see for example Fig 4 B) The degeneration occurs within a spindle shaped area and in addition extends further dorsomedially to cover the field just

In cat K 95 (survival period 73 days) the thionine sections (Fig 2 A) show that there is a subtotal hemisection of the 1st sacral segment Part of the ventral column medially and a superficial portion of the ventrolateral quadrant have been spared

In segments rostral to the lesion (Figs 2 B and 2 C), except the first cervical there are two separate contingents of degenerating fibres both situated peripherally *one at the dorsal extremity of the lateral column* one more ventrally The dorsal contingent is restricted to the region just lateral to the root entry zone In the 1st cervical segment however, this contingent is almost absent On close study degenerating fibres can be detected around the cells of the lateral cervical nucleus The ventral contingent of peripheral degeneration in segments rostral to the lesion seems to extend somewhat more dorsally than the corresponding contingent in cat K 78 (cp Fig 2 C with Figs 1 C and D)

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In the *13th thoracic* segment (Fig 3 B) degeneration on the left side is found mainly peripherally *within the field corresponding to the lesion*

Within the dorsolateral quadrant the degeneration is restricted to the ventral portion whereas the dorsal part is entirely free

In the *10th and 7th thoracic* segments *the degeneration has moved dorsally* Its dorsal limit is found considerably dorsal to the level of the caput of the dorsal horn There is however no degeneration in the field just lateral to root entry zone

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area at the periphery of the dorsolateral quadrant and in the dorsal part of the field just lateral to the root entry zone In the 1st cervical segment the degeneration is seen dorsal to that invagination of the surface of the lateral column which is located about the level of the central canal

It will be documented below that the field just lateral to the root entry zone contains in addition to the fibres described moving into it from more ventrally at levels above the caudal end of the column of Clarke fibres which can be traced from levels below this These latter fibres are seen just lateral to the root entry zone from immediately rostral to a lesion of this part of the cord

Before considering this fibre component however the configuration of the field lateral to the root entry zone will be described

In addition to *cats K 59 61 68 71, 75 and 77* just described as well as five other cases (cats *K 97, 99, 100, 122 and 137* survival periods from 4 to 63 days) with lesions of the dorsal part of the lateral column at levels between the 6th and 1st lumbar segments demonstrate that the field of degeneration between the root entry zone and the dorsal aspect of the area of the lateral corticospinal tract does not have the same shape at different spinal levels In the segments below the 2nd thoracic (the conditions caudal to the thoracic cord were not especially investigated) the field has a somewhat semilunar shape (Fig 6 A) It extends along the lateral border of the root entry zone from the apex of the dorsal horn towards the periphery of the cord where it is continuous with the spindle shaped field mentioned above (cp Fig 4 B)

In the upper two thoracic and lower two or three cervical segments (Fig 6 B) where the dorsal horn protudes towards the periphery of the cord the field is pushed dorsolaterally Its ventral extremity is still in close lateral apposition to the *apex of the horn*

From the 6th or 5th up to the 2nd cervical segment (Fig 6 C see also Fig 4 B) the ventral extremity of the area of *degeneration* is still situated lateral to the apex of the dorsal *horn* but since in these segments this horn is further removed from the surface than in the segments just described the field of *degeneration* extends again more deeply In contrast to the semilunar elongated appearance characteristic for the field below the 2nd thoracic segment the area is considerably broadened

In the 1st cervical segment the ventral part of the area of degeneration just described cannot be recognized (cp Figs 4 B and 4 C)

In *cat K 9*5 reported above (Fig 2) as well as in *cats K 99 and 100*

lateral to the root entry zone In none of these cases does the degeneration extend to the region corresponding to the ascending fibres of the ventrolateral quadrant from more caudal levels In the 1st cervical segment the degenerate fibres are located dorsal to the invagination of the surface, which is seen about the level of the central canal (Figs 4 C and 5 B)

The evaluation of the ascending degeneration in these cases, especially the possible existence of a dorsal shift of the fibres, is complicated by the presence of an ascending fibre component which as will be documented below, probably passes to the lateral cervical nucleus However, a case (cat K 102) in which there is concomitant damage to the contralateral grey matter with an ascending degeneration probably resulting from this, gives information of value and will be considered in this section

That a great part of the degeneration found within the spindle shaped area and the field just lateral to the root entry zone is due most probably to damage to the column of Clarke is demonstrated by the following case

Cat K 102 (survival period 35 days) has a lesion at the level of the 2nd lumbar segment (Fig 5 A) In addition to a lesion of the lateral and ventral columns on the left side which encroaches upon the ventral horn there is a lesion of the dorsal columns extending into the grey matter dorsal to the central canal This latter lesion encroaches upon the column of Clarke on the right side (possibly also on that on the left) The degeneration on the right side will be described here

In the *10th thoracic* segment on the right side (Fig 5 B) degenerating fibres are seen in the dorsolateral quadrant occupying approximately its outer third The dorsal portion of the quadrant is practically free from degenerating fibres

In the *6th thoracic* segment (Fig 5 C) most of the fibres are found within a spindle shaped peripheral zone The dorsomedially situated fibres are seen closer to the root entry zone than in the 10th thoracic segment

In the *1st thoracic* segment (Fig 5 D) the spindle shaped peripheral zone is more discrete and the degenerating fibres covering it are more closely packed In addition some fibres are seen just lateral to the root entry zone

Essentially the same findings as in cat K 102 on the right side are made in *cat K 79* (mentioned above), on the side contralateral to the incision Presumably this degeneration in both cases is due to damage to the column of Clarke contralateral to the incision although in cat K 79 no damage to this could be ascertained in thionine sections at the level of the incision The degenerating fibres turn peripherally and dorsomedially along the periphery of the cord in their ascending course In the upper cervical segments they are found within the spindle shaped

area at the periphery of the dorsolateral quadrant and in the dorsal part of the field just lateral to the root entry zone In the 1st cervical segment the degeneration is seen dorsal to that invagination of the surface of the lateral column which is located about the level of the central canal

It will be documented below that the field just lateral to the root entry zone contains in addition to the fibres described moving into it from more ventrally at levels above the caudal end of the column of Clarke fibres which can be traced from levels below this These latter fibres are seen just lateral to the root entry zone from immediately rostral to a lesion of this part of the cord

Before considering this fibre component however the configuration of the field lateral to the root entry zone will be described

In addition to cats K 59 61 6? 71 75 and 77 just described as well as five other cases (cats K 97, 99, 100 122 and 137 survival periods from 4 to 63 days) with lesions of the dorsal part of the lateral column at levels between the 6th and 1st lumbar segments demonstrate that the field of degeneration between the root entry zone and the dorsal aspect of the area of the lateral corticospinal tract does not have the same shape at different spinal levels In the segments below the 2nd thoracic (the conditions caudal to the thoracic cord were not especially investigated) the field has a somewhat semilunar shape (Fig 6 A) It extends along the lateral border of the root entry zone from the apex of the dorsal horn towards the periphery of the cord where it is continuous with the spindle shaped field mentioned above (cp Fig 4 B)

In the upper two thoracic and lower two or three cervical segments (Fig 6 B) where the dorsal horn protudes towards the periphery of the cord the field is pushed dorsolaterally Its ventral extremity is still in close lateral apposition to the apex of the horn

From the 6th or 5th up to the 2nd cervical segment (Fig 6 C see also Fig 4 B) the ventral extremity of the area of degeneration is still situated lateral to the apex of the dorsal horn but since in these segments this horn is further removed from the surface than in the segments just described the field of degeneration extends again more deeply In contrast to the semilunar elongated appearance characteristic for the field below the 2nd thoracic segment the area is considerably broadened

In the 1st cervical segment the ventral part of the area of degeneration just described cannot be recognized (cp Figs 4 B and 4 C)

In cat K 95 reported above (Fig 2) as well as in cats K 99 and 100

(survival periods 4 and 25 days, respectively) with lesions in the 6th and 5th lumbar segments, respectively, the lesions include the dorsal part of the dorsolateral quadrant caudal to the column of Clarke. Observations in these cats demonstrate that there is a separate contingent of degeneration *originating from segments of the cord below* the caudal level of the column of Clarke (Fig. 2 B). This contingent is located within the area lateral to the root entry zone and can be traced *up to the 2nd cervical segment*. From about the 6th cervical segment it has a ventral position within the field just described (cp. Fig. 6 C). It seems to terminate, at least partly, in the lateral cervical nucleus since degenerating fibres are seen surrounding the cells of this nucleus and, moreover, only extremely few fibres are visible peripherally and dorsally in the 1st cervical segment. Supporting evidence comes from *cats K 77 and 147* (survival periods 5 and 7 days, respectively) with lesions at the transition between the 1st lumbar and 13th thoracic and in the 3rd lumbar segments, respectively. In these cases at high cervical levels degenerating fibres are likewise present chiefly in the ventral part of the field lateral to the root entry zone (Fig. 6 C). Part of these fibres, at least, seem to terminate in the lateral cervical nucleus.

Discussion

Methods

The extent of a lesion as demonstrated in serial transverse sections stained with thionine and in some cases also according to van Gieson does not exhibit exactly the degeneration as demonstrated in Swank-Davenport preparations. It is seen from the findings of cat K 79 and has been seen in other cases which have not been described here. In some cases there is ascending degeneration on the side contralateral to the incision although the thionine and van Gieson sections may not show any alterations either in the contralateral white matter or in the grey matter on either side. It is possible that sections from parts of the cord adjacent to the segment in which the incision was made might have demonstrated alterations corresponding to the contralateral degeneration visualized in the Swank-Davenport preparations. This was, however, not investigated in this study.

The Swank-Davenport preparations in this material are generally very little contaminated with Marchi artefacts such as the well known "Marchi dust" and impregnation of normal myelin sheaths often described especially along the extreme periphery of the cord. The present

material does not permit conclusions regarding the reason for this fact but it may be possible that the perfusion fixation method used in this study is a factor of importance since it is well known that traumatization of nervous tissue at dissection is apt to produce Marchi artefacts It is true that SWANK and DAVENPORT (1935 b) advised against the use of perfusion fixation in their method but they did not use normal osmotic solutions and suggested that oedema might have been a factor of importance in producing the "Marchi dust" (The artefacts in Marchi preparations have recently been extensively discussed by MARION C SMITH (1956))

In most cases presented above the survival periods are short about 5 days Degenerating fibres are however clearly visible in all cases. Interest in the present study is focused upon the spinocerebellar tracts As the fibres composing these are known to be coarse (FLECHSIG 1876 THIELE and HORSLEY 1901 and others) and since coarse fibres degenerate faster than fine ones (VAN CREVEL 1958) the use of short survival periods may be advantageous However the results obtained in animals with survival periods up to 73 days correspond essentially to those in animals with short survival periods

To refer the distribution of degeneration situated at the periphery of the cord to a level through the central canal cannot be regarded as an exact method because of possible distortion of the spinal cord before fixation This source of error however ought to be negligible in this material where the perfusion fixation has been used and distortion of the spinal cord eliminated

Relations between ascending degeneration and spinocerebellar tracts

The purpose of the analysis of the ascending degeneration in the spinal cord reported above has been to provide a basis for an evaluation of spinal cord lesions with regard to their involvement of dorsal and ventral spinocerebellar tract fibres respectively The analysis shows that at least three ascending contingents of degenerating fibres can be recognized

One contingent is seen at the periphery of the ventral part of the lateral column It can be traced from levels as far caudally as the 1st sacral segment and is present in all segments of the spinal cord more rostrally Its dorsal limit is not the same at various levels of the spinal cord This contingent seems to be composed to a great extent of fibres of the ventral spinocerebellar tract Thus the fibres are of a rather

(survival periods 4 and 25 days respectively) with lesions in the 6th and 5th lumbar segments respectively *the lesions include the dorsal part of the dorsolateral quadrant caudal to the column of Clarke Observations in these cats demonstrate that there is a separate contingent of degeneration originating from segments of the cord below the caudal level of the column of Clarke (Fig 2 B)* This contingent is located within the area lateral to the root entry zone and can be traced up to the 2nd cervical segment *From about the 6th cervical segment it has a ventral position within the field just described* (cp Fig 6 C) *It seems to terminate at least partly in the lateral cervical nucleus* since degenerating fibres are seen surrounding the cells of this nucleus and moreover only extremely few fibres are visible peripherally and dorsally in the 1st cervical segment Supporting evidence comes from *cats K 77 and 147* (survival periods 5 and 7 days *respectively*) *with* lesions at the transition between the 1st lumbar and 13th thoracic and *in the 3rd lumbar segments respectively In these cases at high cervical* levels degenerating fibres are likewise present chiefly in the ventral part of the field lateral to the root entry zone (Fig 6 C) Part of these fibres *at least seem to terminate in the lateral cervical nucleus*

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The Swank–Davenport preparations in this material are generally very little contaminated with Marchi artefacts such as the well known "Marchi dust" and impregnation of normal myelin sheaths often described especially along the extreme periphery of the cord The present

material does not permit conclusions regarding the reason for this fact but it may be possible that the perfusion fixation method used in this study is a factor of importance since it is well known that traumatization of nervous tissue at dissection is apt to produce Marchi artefacts. It is true that SWANK and DAVENPORT (1935 b) advised against the use of perfusion fixation in their method but they did not use normal osmotic solutions and suggested that oedema might have been a factor of importance in producing the "Marchi dust". (The artefacts in Marchi preparations have recently been extensively discussed by MARION C. SMITH (1956).)

In most cases presented above the survival periods are short, about 5 days. Degenerating fibres are, however, clearly visible in all cases. Interest in the present study is focused upon the spinocerebellar tracts. As the fibres composing these are known to be coarse (FLECHSIG 1876, THIELE and HORSLEY 1901, and others), and since coarse fibres degenerate faster than fine ones (VAN CREVEL 1958), the use of short survival periods may be advantageous. However, the results obtained in animals with survival periods up to 73 days correspond essentially to those in animals with short survival periods.

To refer the distribution of degeneration situated at the periphery of the cord to a level through the central canal cannot be regarded as an exact method because of possible distortion of the spinal cord before fixation. This source of error, however, ought to be negligible in this material where the perfusion fixation has been used and distortion of the spinal cord eliminated.

Relations between ascending degeneration and spinocerebellar tracts

The purpose of the analysis of the ascending degeneration in the spinal cord reported above has been to provide a basis for an evaluation of spinal cord lesions with regard to their involvement of dorsal and ventral spinocerebellar tract fibres, respectively. The analysis shows that at least three ascending contingents of degenerating fibres can be recognized.

One contingent is seen at the periphery of the ventral part of the lateral column. It can be traced from levels as far caudally as the 1st sacral segment and is present in all segments of the spinal cord more rostrally. Its dorsal limit is not the same at various levels of the spinal cord. This contingent seems to be composed to a great extent of fibres of the ventral spinocerebellar tract. Thus the fibres are of a rather

coarse diameter This is in agreement with the observations of THIELE and HORSLEY (1901) and others, using neuroanatomical methods and also with OSCARSSON's (1956) neurophysiological investigations The superficial position of the fibres lends some support to the assumption *that they belong to the ventral spinocerebellar tract* (see, for example, BRUCE 1910) The argument carrying most weight is, however, that lesions involving the area in question give rise to degenerating fibres in the cerebellum as will be described in Chapter II Furthermore, the degeneration has a distribution which differs from that ensuing from a lesion of the spinal cord above the caudal end of the column of Clarke, involving the peripheral area dorsal to the one under consideration *The contingent of ascending degeneration situated in the ventrolateral quadrant at lower levels of the cord will, therefore, in the following be taken to represent the ventral spinocerebellar tract*

The second contingent of ascending degeneration covers a spindle shaped field in the dorsolateral quadrant and is connected dorsomedially with an area of degeneration just lateral to the root entry zone As will be documented below, parts of these fields harbour fibres belonging *to the dorsal spinocerebellar tract In the upper cervical segments it* seems justified to distinguish between a ventral and a dorsal part of the field just lateral to the root entry zone Evidence is given for the *assumption that the ventral part of this field is composed of fibres* originating below the caudal end of the column of Clarke These fibres seem to terminate partly at least in the lateral cervical nucleus Evidence will be given in Chapter II for the assumption that lesions in*volving this contingent of fibres at levels below* the column of Clarke do not give rise to degenerating fibres passing to the cerebellum The peripheral part of the dorsolateral quadrant dorsal *to the ventral spino*cerebellar tract in segments above the caudal end of the column of Clarke harbours fibres belonging to the dorsal spinocerebellar tract This is in agreement with *the findings of authors having studied the as*cending degeneration following lesions of the column of Clarke (BRUCE 1910, PASS 1933) Furthermore, most of the fibres are seen to be rather coarse confirming previous anatomical (FLECHSIG 1876 and others) as well as physiological (GRUNDFEST and CAMPBELL *1942 LAPORTE* and LUNDBERG 1956) studies Finally, lesions *involving this part of* the dorsolateral quadrant will give rise to degenerating fibres ter*minating in the cerebellum* and distributed in a somewhat different manner from those of the ventral spinocerebellar tract (see Chapter II) The degeneration in the dorsolateral quadrant considered here will be taken to represent the dorsal spinocerebellar tract

If the dorsal part of the field just lateral to the root entry zone in the upper cervical segment really harbours dorsal spinocerebellar tract fibres cannot be definitely ascertained but seems very probable especially on the basis of the findings in cat K 102 (see Fig 5)

Fibre course

As discussed above it is very likely that most of the degenerating fibres within the ventrolateral field belong to the ventral spinocerebellar tract Likewise it is probable that the great bulk of the degenerating fibres at the periphery dorsal to the ventral spinocerebellar tract belong to the dorsal spinocerebellar tract Part of the degenerating fibres in the dorsolateral quadrant are however probably afferent fibres to the lateral cervical nucleus Some of the fibres may be spinal afferents to the vestibular nuclei (POMPEIANO and BRODAL 1957 a) but their quantity appears to be small

From an analysis of the present cases it is seen that there is a dorsal shift of fibres from the lumbosacral *ventrolateral* quadrant (representing the ventral spinocerebellar tract) during their ascending course through the thoracic and lower cervical cord In the upper cervical segments however the fibres return to a more ventral position No transference of fibres from the ventrolateral quadrant to the dorsal portion of the dorsolateral quadrant can however be demonstrated In all cases where degeneration is found in this dorsal portion at high levels of the cord degenerating fibres are also found just above the lesion within either the peripheral or deep portion of the lateral column These latter degenerating fibres lie more dorsal than the ascending fibres of the ventral spinocerebellar tract from more caudal levels

A shift of peripheral fibres from the ventrolateral into the dorsolateral quadrant during their rostral course through the spinal cord has been reported by MARBURG (1903) and MARION C SMITH (1957) in man and by BRUCE (1910) in the monkey Similar observations do not appear to have been made in the cat BECK (1927) referring to his Figs 5 and 6 reports that in his cat 2 with a lesion in the 1st lumbar segment he observed that the "ventral spino cerebellar tract" had moved somewhat dorsally in the 1st cervical segment as compared with the 6th thoracic segment Even if it is evident that his Fig 5 does not illustrate the 6th thoracic segment but probably one of the lower cervical segments a comparison reveals contrary to Beck's text

a ventral shift of the fibres composing the tract in question thus in accord with the results obtained in the present material It is also worth noticing that the "*ventral spino cerebellar tract*" in Beck's Fig extends dorsally to a level somewhat dorsal to the central canal This is in accordance with the results in the cases with low ventro lateral lesions in this study

In the present investigation no evidence has been found for a transference of fibres from the ventrolateral quadrant to the *dorsal* part of the dorsolateral quadrant as reported by BRUCE (1910) and MARION C SMITH (1957) MARBURG (1903) seems to have been of the same opinion as Bruce and Marion C Smith since he considered those ventral spinocerebellar fibres which pass into the field of the dorsal spino cerebellar tract to intermingle with the fibres of that tract These differences may perhaps be due to species variations Considering the results reported by Bruce it is however of interest to note that he mentions (p 398) that the "transference of fibres from the ventral cerebellar tract to the dorsal cerebellar tract is seen in all cases where the lesion is made in any of the lower thoracic segments If however the lesion be in the upper thoracic region the transference of fibres from the one tract to the other does not take place nearly to the same extent This might indicate that the lesions in his material encroached upon the ventralmost portion of the dorsolateral quadrant since in the present investigation it has been demonstrated that in the upper thoracic segments the fibres of the ventral spinocerebellar tract transected in the ventrolateral quadrant at low spinal levels are localized more dorsally and are probably not intermingled here with fibres of the dorsal spinocerebellar tract A slight encroachment upon the dorso lateral quadrant in the upper thoracic region would then be expected to give rise to less degeneration in the dorsal spinocerebellar tract than a lesion at low thoracic levels The results obtained in the present study are in accordance with those of YOSS (1953) In the marchi technique he demonstrated that the fibres composing what he defines as the ventral spino cerebellar tract turn dorsally along the periphery of the cord up to the cervical enlargement Rostral to this there is a return to a more ventral position In a case with a lesion in the 3rd sacral segment he reports that the dorsalmost component of the degenerating fibres in the 7th cervical segment extends dorsally slightly into the dorsolateral column as this has been defined in the present study Yoss did not however investigate in more detail the dorsal extent of the ventral spinocerebellar tract

It is also of interest in this connection that WHITLOCK's (1952) Fig 4

shows that in the pigeon the ventral spinocerebellar tract in the lower cervical cord is situated definitely dorsal to the level of the central canal thus indicating that the condition in birds may be similar to that in mammals although he does not comment on this finding. It is in agreement with the findings in the present investigation that HOLMQVIST and OSCARSSON (personal communication, 1961) in electrophysiological studies have found a dorsal shift of ventral spinocerebellar fibres activated from the hind limb in the cat. They compared the level of the 3rd to 2nd cervical segments with the 1st lumbar.

With regard to the degeneration within the dorsolateral quadrant mention was made above of a bundle of fibres lying in close apposition to the dorsal root entry zone. This may to some extent be mixed with fibres of the dorsal spinocerebellar tract. This bundle seems to consist chiefly of fibres originating from levels below the caudal *end of the column of Clarke*. It can be traced rostrally to the level of the lateral cervical nucleus described by REXED and BRODAL (1951). Degenerating fibres can be seen surrounding cells of this nucleus. In the 1st cervical segment the bundle has disappeared. These *facts indicate* the termination of the fibres in the lateral cervical nucleus. The present findings are in complete agreement with those *of BRODAL and REXED* (1953) who in silver impregnated sections found degenerating terminal fibres and boutons in the lateral cervical nucleus following lesions of the cord even with lesions below the level of the column of Clarke. *Their* assumption that the lateral cervical nucleus is largely supplied by spinal afferents separate from those of the dorsal *spinocerebellar tract* is confirmed in the present study. It may be *mentioned that* LEWANDOWSKY (1904) even if he was not aware of the fact that the *lateral* cervical nucleus is a separate nucleus but regarded *it as a portion* of the lateral reticularis nucleus, was of the opinion *that the nucleus* received fibres from the lumbosacral cord.

In the present investigation it is shown that the *bundle of fibres* which is probably composed of afferents to the lateral cervical nucleus in rostral segments from about *the 6th cervical has a marked* ventral position within the field *just lateral to the root* entry zone. Whether the fibres to the lateral cervical nucleus *coming from levels* above the caudal end of the column of Clarke have a somewhat different position cannot be decided.

It appears that the fibres within the *ventral* spinocerebellar tract turn peripherally as they ascend the cord and that fibres from caudal levels are found more dorsally *than fibres from* more rostral levels. This arrangement is *in agreement* with the classical notions of the

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Chapter II

Terminal distribution of spinocerebellar tracts

In the previous chapter it has been demonstrated that the fibres composing the ventral spinocerebellar tract turn dorsally during their ascending course. In the rostral cervical segments, however, they change their position once more and move ventrally. Even if a small zone of overlapping may exist, it must be concluded that the fibres of the ventral spinocerebellar tract do not intermingle to any considerable extent with those of the dorsal one. The dorsal limit of the ventral spinocerebellar tract as determined in the previous section will therefore be used as a criterion in the selection of cases suitable for tracing the course of dorsal and ventral *spinocerebellar tracts separately in the* cerebellum. The criteria used in the identification of degenerating structures in the cerebellum have been described elsewhere (BRODAL and GRANT 1962). Quantitative evaluations of degeneration have been made only where striking differences have appeared consistently in successive sections. The lateral boundaries of regions showing *degeneration* have been estimated mostly by examining successive sagittal sections. In some cases transverse serial sections through some folia *from the* anterior lobe have been used for the same purpose.

The terminology of LARSELL (1953) will be used for the cerebellar lobules and fissures. LARSELL (personal *communication* 1961) has recently subdivided his lobule VIII *in the* cat into two parts, sublobules VIII A and VIII B, separated by the intrapyramidal fissure, as indicated in the drawings. To facilitate the description, the term "posterior vermis" will be used collectively for the vermal lobules VII *to* X. The modified Nauta method, *in which the Laidlaw* solution is used, will be referred to as the Nauta-Laidlaw method.

Results

The results will be presented under the *following* headings:

A. Total cerebellar distribution of spinocerebellar tracts
B. Cerebellar distribution of dorsal spinocerebellar tract
C. Cerebellar distribution of ventral spinocerebellar tract

general arrangement of the fibres in the ventrolateral column (WEAVER and WALKER 1941, YOSS 1953, and others)

The fibres of the dorsal spinocerebellar tract present a similar arrangement The findings in cat K 102 (Fig 5) demonstrate a shift towards the periphery and dorsally along it of fibres ascending the cord This pattern is in agreement with the findings of SHERRINGTON and LASLETT (1903) and YOSS (1952)

The shift of the fibres in the dorsolateral quadrant in cat K 102, most probably originating in the column of Clarke, is in complete agreement with findings of PASS (1933) From his Figs 13, 14 and 15 a dorsal shift of fibres from the column of Clarke is quite evident although this fact is not commented upon by the author

It is of interest that the 1st cervical segment appears to be particularly suited to evaluate spinocerebellar degeneration The invagination of the surface of the lateral column at the level of the central canal seems to coincide with the limit between the area occupied by the two spinocerebellar tracts

Some authors have described an intermediate spinocerebellar tract (PELLIZI 1895, BECK 1927, ANDERSON 1943, and others), situated in the lateral column between the areas occupied by the dorsal and ventral spinocerebellar tracts Whereas according to ANDERSON (1943) the fibres of the intermediate tract appear to be distributed with the ventral spinocerebellar tract, according to BECK (1927) they seem to be more closely associated with the dorsal spinocerebellar tract

The area within which previous authors have described fibres of the intermediate spinocerebellar tract appears to correspond to the "head" of the comma shaped field which is seen in Fig 1 This "head" seems to be nothing but the dorsalmost region of the ventral spinocerebellar tract In the 1st cervical segment it seems, however, that the ventral most portion of the dorsal spinocerebellar tract has a medially directed contingent (Fig 4 C), which thus contributes to the formation of this "head". No conclusions concerning the further course of these fibres can be drawn from the present material

culus are mainly of a smaller calibre than those in the anterior lobe and sublobule VIII B (cp. Figs. 8 A and B).

In this case the lateral parts of the dorsal paraflocculus together with the ventral paraflocculus and the flocculus on the two sides have been separated from the rest of the cerebellum and cut serially. No degenerating fibres are seen in any of these sections nor in sections from the ansiform lobules. Although degenerating fibres are seen to course through the fastigial nuclei no degenerating fibres can be seen to terminate in the cerebellar nuclei.

B. Cerebellar distribution of dorsal spinocerebellar tract

The cerebellar distribution of the dorsal spinocerebellar tract has been investigated in 13 cases.

Nine animals (*K 61, 68, 70, 71, 77, 159, 170, 175, 181*; survival periods from 4 to 11 days) with similar lesions which include the dorsal portion of the dorsolateral quadrant at levels between the 3rd lumbar and 3rd cervical segments present degenerating fibres in lobules II, III, IV and the anteriormost portion of lobule V, ipsilaterally. In seven of these degeneration is seen in lobule I, but here the degenerating fibres seem to be less conspicuous than in the lobules just posterior to lobule I. In all of the nine cases there are degenerating fibres coursing to the ipsilateral sublobule VIII B and in seven of them where this can be investigated fibres can be traced to the ipsilateral paramedian lobule and medialmost portion of the dorsal paraflocculus. Within the paramedian lobule the degenerating fibres are seen in the *pars copularis*, although sometimes some degeneration can be detected in the most posterior portion of the pars posterior as well. Degenerating fibres to the most anterior portion of the *anterior folium* of the pars anterior are seen in some cases, but they seem to be extremely sparse. In six of the nine cases degenerating fibres can be seen within the anterior lobe contralaterally. In one of the nine cases, *cat K 175* (survival period 4 days), with a restricted lesion including the dorsalmost portion of the dorsolateral quadrant and extending to the level of the cervix of the dorsal horn in the 3rd cervical segment, there are, however, practically no degenerating fibres contralateral to the lesion. In five of the cases with degeneration in the contralateral anterior lobe the fibres are distributed mainly within *lobule II* and almost exclusively within a sagittal zone at the level of the lateral extremity of lobule I. In all of these five cases the lesions extend ventrally as far as the level of the transition between the ventral and dorsal spinocerebellar tracts as defined in Chapter I.

In all cases with lesions of the dorsal spinocerebellar tract the areas of distribution within the *anterior lobe*, sublobule VIII B and the

1. *Total cerebellar distribution of spinocerebellar tracts*

The total cerebellar distribution of both spinocerebellar tracts has been investigated in 4 cases with unilateral lesions of the lateral columns. The observations in three of the animals (*K 81, 89 and 101*, survival periods 62, 31 and 15 days respectively) with unilateral lesions completely destroying the lateral columns at a high cervical level without encroaching upon the lateral cervical nucleus confirm the observations made in the fourth animal (*K 153*) which will be described here in detail.

Cat K 153 (survival period 5 days). An incision involving the areas of the dorsal and the ventral spinocerebellar tracts was made on the left side in the 3rd cervical segment. The ventral column and a minute portion of the ventrolateral quadrant have been spared (Fig. 9 A). Some of the caudalmost cells of the lateral cervical nucleus are included in the lesion. The degeneration in a section from the caudalmost part of the medulla is seen in Fig. 9 B.

The degeneration in the cerebellum is shown in Fig. 10 C. Degeneration is found within the anterior lobe, Larsell's lobule VI, sublobule VII B, lobules VIII and IX as well as in the paramedian lobule and the medialmost portion of the dorsal paraflocculus. Bilaterally within the anterior lobe there is heavy degeneration in lobules II, III, IV and the most anterior part of lobule V which is hidden in the fissura intraculminata 1. More posteriorly in lobule V degeneration is extremely sparse contrasting sharply against the heavy degeneration more anteriorly (Fig. 10 B). *Within lobule I there is sparse degeneration. Within lobule VI degeneration* is found only anteriorly in those folia which are hidden in the fissura prima and which have been labelled f and d by Larsell. Here degenerating fibres are as scanty as in the posterior part of lobule V.

Within the posterior vermis degenerating fibres are found in sublobule VII B posteriorly and in lobule VIII (Figs. 10 C and 10 B). No degeneration is visible in sublobule VII A and lobule X. Within lobule IX degenerating fibres are found exclusively in the basal and anterior part of the lobule which is hidden in the fissura secunda. Within sublobules VII B and VIII A the degeneration is extremely scanty and contrasts sharply against the heavy degeneration in sublobule VIII B on the left side. On the right there is very sparse degeneration in sublobule VIII B.

Degeneration is found bilaterally in the paramedian lobule (Fig. 10 C). The degeneration on the right side is, however, scanty and contrasts against the heavy degeneration in the left paramedian lobule. Within the paramedian lobules degeneration is furthermore confined to the pars copularis, to the basal and posterior part of the pars posterior facing the pars copularis and to the anterior half of the most anterior folium of the pars anterior facing the fissura ansoparamedianus (Figs. 10 C and A).

Sagittal sections through the paramedian lobule will also include some folia posterior to it (see Fig. 10 A). These constitute the medialmost portion of the dorsal paraflocculus (LARSELL, personal communication 1961). Heavy degeneration is found within these folia on the left side while extremely sparse degeneration is visible on the right.

The degenerating fibres within the paramedian lobule and the dorsal paraflоc-

culus are mainly of a smaller calibre than those in the anterior lobe and sublobule VIII B (cp Figs 8 A and B)

In this case the lateral parts of the dorsal paraflocculus together with the ventral paraflocculus and the flocculus on the two sides have been separated from the rest of the cerebellum and cut serially No degenerating fibres are seen in any of these sections nor in sections from the ansiform lobules Although degenerating fibres are seen to course through the fastigial nuclei no degenerating fibres can be seen to terminate in the cerebellar nuclei

B *Cerebellar distribution of dorsal spinocerebellar tract*

The cerebellar distribution of the dorsal spinocerebellar tract has been investigated in 13 cases

Nine animals (K *61, 68, 70 71 77 159 170 175 181* survival periods from 4 to 11 days) with similar lesions which include the dorsal portion of the dorsolateral quadrant at levels between the 3rd lumbar and 3rd cervical segments present degenerating fibres in lobules II III IV and the anteriormost portion of lobule V ipsilaterally In seven of these degeneration is seen in lobule I but here the degenerating fibres seem to be less conspicuous than in the lobules just posterior to lobule I In all of the nine cases there are degenerating fibres coursing to the ipsilateral sublobule VIII B and in seven of them where this can be investigated fibres can be traced to the ipsilateral paramedian lobule and medialmost portion of the dorsal paraflocculus Within the paramedian lobule the degenerating fibres are seen in the pars copularis although sometimes some degeneration can be detected in the most posterior portion of the pars posterior as well Degenerating fibres to the most anterior portion of the anterior folium of the pars anterior are seen in some cases but they seem to be extremely sparse In six of the nine cases degenerating fibres can be seen within the anterior lobe contralaterally In one of the nine cases *cat K 175* (survival period *4 days*) *with* a restricted lesion including the dorsalmost portion of the dorsolateral quadrant and extending to the level of the cervix of the dorsal horn in the 3rd cervical segment there are however practically no degenerating fibres contralateral to the lesion In five of the cases with degeneration in the contralateral anterior lobe *the fibres* are distributed mainly within lobule II and almost exclusively within a sagittal zone at the level of the lateral extremity of lobule I In all of these five cases the lesions extend ventrally as far as the level of the transition between the ventral and dorsal spinocerebellar tracts as defined in Chapter I

In all cases with lesions of the dorsal spinocerebellar tract the areas of distribution within the anterior lobe sublobule VIII B and the

paramedian lobule as well as the medialmost portion of the dorsal paraflocculus, are the same regardless of the level of the lesion provided the lesion is situated above the 3rd lumbar *segment* This is demonstrated by a comparison between the findings in *cats K 71, 77, 170 and 181* (survival periods 5, 5, 8 and 11 days respectively) with lesions in the upper lumbar region (cat K 77 has a lesion at the transition between the 1st lumbar and 13th thoracic segment), and the findings in *cats K 68, 152 and 175* (survival periods 5, 11 and 4 days respectively) with lesions in the cervical cord below the level of the lateral cervical nucleus

Whether there is a differential distribution in the transverse plane of the fibres within the anterior lobe cannot be decided from the present material However, from the study of the sagittal sections the impression is gained that degenerating fibres are somewhat more abundant in sections somewhat lateral to the median plane, approximately at the level of the lateral extremity of lobule I Observations in transverse serial sections through the anterior lobe, available in *cat K 75* (survival period 5 days) with a lesion in the upper lumbar region *seem to support this impression*

Cats K 99, 171 and 180 (survival periods 1 19 and 11 days respectively) are cases with dorsolateral quadrant lesions below the caudal end of the column of Clarke in the 6th (K 99) and 5th (K 171, 180) lumbar segments No degenerating fibres are found ipsilaterally in the cerebellum

To illustrate the distribution of the dorsal spinocerebellar tract in the cerebellum the following case (K 68) with a lesion above the rostral end of the column of Clarke will be described in detail

Cat K 68 (survival period 5 days) An incision was made in the 6th cervical segment on the left side The lesion (Fig 11 A) comprises the dorsal portion of the dorsolateral quadrant and extends somewhat ventral to the level of the cervix of the dorsal horn Thus it probably includes most or all fibres of the dorsal spinocerebellar tract In addition the lesion encroaches upon the dorsal horn as well as the dorsal columns

The resulting degeneration in a Swank-Davenport preparation from the 1st cervical segment is shown in Fig 11 B Degenerating fibres are found collected peripherally within the dorsolateral quadrant from just lateral to the dorsal horn and extending ventrally

The distribution of degenerating fibres within the cerebellum is shown in Fig 11 C Degeneration is found mainly on the side ipsilateral to the lesion but some degeneration is also seen on the contralateral right side The degeneration on the left side will be described first

On the left side degeneration is found in the anterior lobe lobules VI VIII and sublobule VII B in the paramedian lobule and in the medial portion of the dorsal

paraflocculus Within the anterior lobe the degenerating fibres are seen in lobules I to V The degeneration within lobule I however seems to be more sparse than in the lobules immediately posterior to it Within lobule V degeneration is heavy in the anteriormost portion facing the fissura intraculminata I More posteriorly, as well as in lobule VI there are only a few scattered fibres Within lobules II III and IV there is degeneration of about the same intensity as in the most anterior portion of lobule V Degeneration appears to be more heavy in lobules II and III than in lobule IV but this cannot be definitely settled The degeneration in the anterior lobe on the left side is present in sections from the midline to the left extremity of all the lobules mentioned Within lobules II to V degeneration seems however to be particularly heavy in the (sagittal) sections passing through the left extremity of lobule I

Within the posterior vermis on the side of the lesion numerous degenerating fibres are found in sublobule VIII B while only a few scattered fibres are visible in sublobules VIII A and VII B

Within the paramedian lobule on the left side degenerating fibres are found in the pars copularis These fibres as well as those visible in the medialmost portion of the dorsal paraflocculus have a smaller calibre than those found in other parts of the cerebellum (see Figs 8 A and B) The lateral parts of the paramedian lobule seem to be less densely supplied with degenerating fibres than the central and medial ones

On the contralateral right side some degeneration is found but it is definitely less heavy than on the side of the lesion Within the anterior lobe the degenerating fibres are found with the same antero posterior distribution as on the left side The sections close to the midline contain extremely few fibres as compared with those on the left side and with those in some successive sections at a sagittal level about the right extremity of lobule I In these more lateral sections heavy degeneration is seen in lobule II whereas there is only sparse degeneration within lobules I III IV and the anterior part of V Lateral to the sections mentioned there is sparse degeneration within all of these lobules In lobule VIII on the right side extremely few degenerating fibres are found in sublobule VIII B No degenerating fibres are visible within the contralateral paramedian lobule and the dorsal paraflocculus

4 Cerebellar distribution of ventral spinocerebellar tract

The cerebellar distribution of the ventral spinocerebellar tract has been investigated in 13 cases They comprise cats K 65, 76 79, 80 83 88 93 95 96 97, 100 108 and 160 (survival periods 3 to 73 days) with lesions between the 3rd sacral and 3rd cervical segments

The ventral spinocerebellar tract terminates mainly contralaterally and mainly within the anterior lobe To a lesser extent it terminates in sublobule VIII B in the paramedian lobule and the dorsal paraflocculus and mainly contralaterally

The details of the case (K 83) with the most rostral lesion illustrate these points

Cat K 83 (survival period 6 days) An incision was made in the ventrolateral quadrant on the left side at the level of the 3rd cervical segment The lesion com

paramedian lobule as well as the medialmost portion of the dorsal paraflocculus are the same regardless of the level of the lesion provided the lesion is situated above the 3rd lumbar segment This is demonstrated by a comparison between the findings in *cats K 71, 77 170 and 181 (survival periods 5 5 8 and 11 days respectively)* with lesions in the upper lumbar region (cat K 77 has a lesion at the transition between the 1st lumbar and 13th thoracic *segment*) and the findings in *cats K 68, 152 and 175* (survival periods 5 11 and 4 days respectively) with lesions in the cervical cord below the level of the lateral cervical nucleus

Whether there is a differential distribution in the transverse plane of the fibres within the anterior lobe cannot be decided from the present material However from the study of the sagittal sections the impression is gained that degenerating fibres are somewhat more abundant in sections somewhat lateral to the median plane approximately at the level of the lateral extremity of lobule I Observations in transverse serial sections through the anterior lobe available in *cat K 75* (survival period 5 days) with a lesion in the upper lumbar region seem to support this impression

Cats K 99, 171 and 180 (survival periods 1 19 and 11 days respectively) are cases with dorsolateral quadrant lesions below the caudal end of the column of Clarke in the 6th (K 99) and 5th (K 171 180) *lumbar segments No degenerating fibres are found ipsilaterally in the* cerebellum

To illustrate the distribution of the dorsal spinocerebellar tract in the cerebellum the following case (K 68) with a lesion above the rostral end of the column of Clarke will be described in detail

Cat K 68 (survival period 5 days) An incision was made in the 6th cervical segment on the left side *The lesion (Fig 11 A) comprises the dorsal portion of the* dorsolateral quadrant and extends somewhat ventral to the level of the cervix of the dorsal horn Thus it probably includes most or all fibres of the dorsal spinocerebellar tract In addition the lesion encroaches upon the dorsal horn as well as the dorsal columns

The resulting degeneration in a Swank Davenport preparation from the 1st *cervical segment* is shown in Fig 11 B *Degenerating* fibres are found collected peripherally within the dorsolateral quadrant from just lateral to the dorsal horn and extending ventrally

The distribution *of degenerating fibres within the cerebellum is shown in Fig 11 C* Degeneration is found mainly on the side ipsilateral to the lesion but some degeneration is also seen on the contralateral right side The degeneration on the left side will be described first

On the left side degeneration is found in the anterior lobe lobules *VI VIII* and sublobule VII B in the paramedian lobule and in the medial portion of the dorsal

the anterior lobe approximately in the plane of the brachia conjunctiva In some of these sections lobule I is seen in continuity with what is probably lobule II In the latter heavy degeneration is visible contralateral to the cord lesion just lateral to a sagittal plane through a lateral extremity of lobule I (Fig 14) The regions medial and lateral to the degeneration in lobule II are almost free from degeneration In one additional case (*cat K 108*) coronal serial sections prepared from the anterior lobe demonstrate that the most medial part is free from degeneration

The degeneration within the anterior lobe does not extend as far posteriorly in cases with lesions of the ventral spinocerebellar tract at a low spinal level as in those with lesions at higher levels

In case K 83 which has already been described in detail and in case *K 160* with a lesion of the ventral spinocerebellar tract in the 3rd thoracic segment heavy degeneration in the anterior lobe extends posteriorly to include the most anterior part of lobule V

In six cases with lesions of the ventral spinocerebellar tract at levels between the 4th and 7th lumbar segments heavy degeneration is found to be strictly confined to lobule II and not to extend more posteriorly This is illustrated in detail in cat K 93

(*Cat K 93* survival period 5 days) The lesion comprises the ventral portion of the ventrolateral quadrant at the level of the 5th lumbar *segment The heavy* degeneration in the anterior lobe is found to extend somewhat more posteriorly than in the previous case including the most posterior *part of lobule II In addition* there is very sparse degeneration in lobules III and IV

In one case (K 96) the lesion is at the level of the 3rd sacral segment and the heavy degeneration is confined to *only* the anterior portion of lobule II The details are as follows

(*Cat K 96* survival period 47 days) In this animal there is a *hemisection* with some encroachment upon the medial *portion of the contralateral* left half of the *cord* at the level of the 3rd sacral segment (Fig 13 A) The degeneration within the anterior lobe is shown in Fig 13 B

There is rather heavy degeneration localized *strictly to the* anterior part of lobule II The main degeneration is *found somewhat* to the left of the median plane about the level of the lateral extremity of lobule I There is *no degeneration* in the anterior lobe posterior to lobule II except for some *extremely* few degenerating fragments in the anterior part of lobule III contralateral to the lesion Extremely few degenerating fibres are visible *in sublobule VIII B* contralateral to the lesion No degeneration is found in the *medial* portion of the dorsal paraflocculi nor in the paramedian lobules

prises almost the entire peripheral part of the ventrolateral quadrant. It does not extend into the dorsolateral quadrant (Fig. 12 A) and does not encroach upon the lateral cervical nucleus. The degeneration in the 1st cervical segment is seen in Fig. 12 B. Degenerating fibres in the cerebellum (Fig. 12 C) are found bilaterally but are definitely more abundant on the right side, i.e. contralateral to the lesion. Here they seem to be most numerous just about a sagittal plane through the right extremity of lobule I.

No striking difference as regards the quantity of degeneration on the two sides is found in lobule I where degeneration is relatively scanty. The bulk of degeneration is found within lobules II to IV. In those sections where the degeneration is most pronounced in these lobules, it extends into the most anterior basal part of lobule V.

Within the posterior vermis some degeneration is found in lobule VIII, most pronounced in sublobule VIII B. Some degeneration is visible in the medialmost portion of the dorsal paraflocculus and in those parts of the paramedian lobule which are supplied with degenerating fibres in cat K 153 (Figs. 9 and 10). Most of the degenerating fibres have a fine calibre.

In ten of the remaining twelve cases with lesions comprising the ventral spinocerebellar tract the degeneration is confined to the regions described in cat K 83. In two cases transverse sections were prepared from the anterior lobe and the other parts of the cerebellum were not investigated.

In nine cases with unilateral lesions of the ventral spinocerebellar tract the degeneration is found to be definitely most pronounced on the side contralateral to the lesion. These nine cats having lesions at levels from the 3rd sacral up to the 3rd cervical segment include only three cases with lesions rostral to the 3rd lumbar segment.

In all cases investigated with lesions of the ventral spinocerebellar tract at levels from the 3rd thoracic segment and more caudally there is only extremely sparse degeneration within sublobule VIII B, the medial portion of the dorsal paraflocculus and within the paramedian lobule mainly contralateral to the lesion. In cat K 83, reported above, the degenerating fibres seem to be more abundant.

Observations in eleven of the thirteen animals confirm the findings reported from cat K 83 that the degenerating fibres in the anterior lobe are most numerous within a strip-like longitudinal zone just lateral to the level of a sagittal plane through the lateral extremity of lobule I. In one of these cases (*cat K 79*)[1] serial sections have been cut through

[1] Cat K [illegible] has been reported in Chapter I to have some degenerating fibres in the dorsolateral quadrants, interpreted as belonging to the dorsal spinocerebellar tracts. In comparison with the degenerating fibres in the ventral spinocerebellar tracts they are, however, very sparse.

1st cervical segment the degenerating fibres within the dorsolateral quadrant are situated along the ventral portion of the periphery and practically no degenerating fibres are found dorsally within the dorsolateral quadrant (Fig 3 F) The dorsal portion at the periphery of the dorsolateral quadrant contains heavy degeneration in the 1st cervical segment in cat K 61 (Fig 4 C) Here the lesion comprises the *dorsal* part of the dorsolateral quadrant in the 7th cervical segment (Fig 4 A)

Discussion

Methods

The aim of the second part of this investigation has been to determine the terminal areas of each of the two spinocerebellar tracts Hemisections of the cord at a high cervical level have been used for the determination of the total distribution of the two spinocerebellar tracts together Thereby it is essential however to make the hemisections below the caudal end of the lateral cervical nucleus since this seems to have efferent connections with the cerebellum (REXED and BRODAL 1951) In all cases it has been checked if the lesions encroach upon this nucleus In attempts to make incisions restricted to only one of the spinocerebellar tracts a knowledge of their individual spinal course is essential Since information on this subject in the literature is incomplete it was deemed necessary as a preliminary step to map the spinal course of the tracts in question The results of this study have been presented in Chapter I While there seems to be agreement concerning the origin of the fibres of the dorsal spinocerebellar tract from the cells of the column of Clarke (FLECHSIG 1876 PICK 1878 PASS 1933 and others) extending caudally to the 3rd or 4th lumbar segment in the cat (REXED 1954) the cells of origin of the ventral spinocerebellar tract have so far not been conclusively identified (see however the physiological study of HUBBARD and OSCARSSON 1961) It may be considered as settled however that segments of the cord caudal to the column of Clarke *contribute* to the ventral spinocerebellar tract (MOTT 1895 BRUCE 1910 YOSS 1953 and others) This is confirmed in the present investigation Rostral to the caudal end of the column of Clarke it is almost impossible to produce spinal lesions comprising only the fibres of *one* of the tracts without encroaching upon fibres of the other

In the present study the cerebella have been cut in sagittal sections which are well suited for the study of the distribution of spinocerebel

It appears that the fibres of the ventrolateral quadrant as they ascend the cord change from a medial to a peripheral position and that they are so arranged that fibres from caudal levels lie more dorsally than fibres from more rostral levels

The change in position of the fibres towards the periphery is seen by a comparison of Figs 1 B and C and Figs 3 B and C

The topical arrangement by which fibres from caudal levels lie more dorsally than fibres from more rostral levels is illustrated by the findings in cats *K 78 and 84* The findings of cat K 78 have already been described, those of cat K 84 are as follows

Cat K 84 (survival period 5 days) has a lesion which comprises the ventrolateral quadrant with sparing of its most dorsal portion (Fig 7 A) The lesion encroaches upon the lateral part of the ventral column and slightly upon the ventral horn The dorsal quadrant is not affected

In the *1st cervical* segment degeneration is found at the periphery of the greater part of the ventrolateral quadrant but not in the most dorsal portion of this quadrant (Fig 7 B)

A comparison between the two cases (K 78 and 84) demonstrates that the lesion in the 6th thoracic segment in cat K 84 (Fig 7 A) does not extend dorsally to include the area containing the most dorsally situated degenerating fibres seen at approximately the same segmental level (the 8th thoracic segment) in cat K 78 (Fig 1 C) On ascending the cord these most dorsal degenerating fibres in cat K 78 (Fig 1 C) seem to be included in the dorsal portion of the ventrolateral quadrant just ventral to the level of the central canal in the 2nd cervical segment (Fig 1 E) The degenerating fibres having ascended to the 1st cervical segment in cat K 84 (Fig 7 B) do not reach so far dorsal as to the level of the central canal (arrow, Fig 7 B)

The fibres above the caudal end of the column of Clarke dorsal to *the fibres ascending from the ventrolateral quadrant* at more caudal levels present a shift from a medial to a peripheral position during their ascending course Furthermore, these fibres dorsal to the ones ascending from the ventrolateral quadrant at more caudal levels are topically arranged

The change in position of the fibres towards the periphery is seen on the right side in cat *K 102* which has *already been described* (Figs 5 B–D) The topical arrangement of the fibres is demonstrated by a comparison of the findings in cats *K 61 and 92* which have been described In cat K 92 the lesion encroaches upon the *ventral* portion of the dorsolateral quadrant in the 1st lumbar segment (Fig 3 A) In the

strate however that the total number of spinocerebellar fibres to the paramedian lobule is appreciable indicating that the finer fibres have not been brought to light with the Marchi method Since there is assumed to be a rough correlation between the diameter of a fibre in the normal and the degenerated state (Marion C Smith 1957) the fibres to the paramedian lobule and the dorsal paraflocculus can be assumed to be of a fine calibre

Cerebellar distribution of spinocerebellar tracts

The termination of spinocerebellar fibres has been demonstrated above to be confined to two cerebellar areas an anterior and a posterior one The *anterior area* comprises the anterior portion of the anterior lobe The distribution within this lobe has a sharp posterior border passing through the most anterior portion of lobule V Posterior to this only extremely few fibres are seen scattered within lobule V and the anterior portion of lobule VI The *posterior area* comprises sublobule VIII B the pars copularis of the paramedian lobule and the medialmost portion of the dorsal paraflocculus situated immediately posterior to the pars copularis Some fibres reach also the *most basal* anterior portion of lobule IX in the depth of the *fissura secunda* as well as the most posterior part of the pars posterior of the paramedian lobule Extremely few fibres have been seen within sublobules VII B and VIII A No fibres have been seen to terminate in the cerebellar nuclei

Spinocerebellar fibres have previously *been traced* to the anterior lobe (Ingvar 1918 Beck 1927 Brodal and Jansen 1941 Whitlock 1952 and several others) to lobule VIII the pyramis (the authors mentioned and others) and to the paramedian lobule (Beck 1927 Anderson 1943) As regards the distribution of the fibres *within the* anterior lobe previous investigations have not demonstrated a *posterior* border as distinct as seen in this material although it has been observed that the posterior part of the culmen receives fewer fibres than the adjacent more anterior portions of the anterior lobe (Ingvar 1918 Beck 1927 Brodal and Jansen 1941 Vachananda 1959) *Conflicting* statements are found in the literature concerning the *termination* of spinocerebellar fibres in lobule I (the lingula) Fibres have been found by Ingvar 1918) Brodal and Jansen (1941) Anderson (1943) Yoss 1953 and others and have been demonstrated *in* this study

In the present study the fibres to the posterior vermis have been shown to terminate mainly in sublobule VIII B while only extremely

lar fibres. In some cases folia from the anterior lobe have been dissected free from the rest of the cerebellum in order to permit a more precise mapping of the distribution of degenerating fibres in the transverse plane. In some cases, in addition, most of the paraflocculus together with the flocculus have been separated from the rest of the cerebellum and cut as separate blocks in a plane parallel to the base of the cerebellum. This has been done because portions of these parts are easily lost if sagittal sections are used. Transverse sections through the cerebella have not been employed since identification of the individual lobules, essential in a study like this, would then be almost impossible.

In a separate investigation (BRODAL and GRANT 1962) it has been demonstrated that the silver methods used in the present study are well suited for a study of degenerating spinocerebellar fibres in the cerebellum. The NAUTA methods (1954, 1957) are especially useful for degenerating fibres, while the GLEES (1946) and REUMONT and LHERMITTE (in *Romeis* 1948) methods are necessary if the terminal fibres and boutons are to be studied. BRODAL and GRANT (1962) furthermore confirmed the findings of some previous authors that the spinocerebellar tracts terminate as mossy fibres. The criteria used for the identification of degenerating fibres in that study have been employed here. Usually the tracing of degenerating fibres gives sufficient information about their termination. In case of doubt resort has been taken to a study of degenerating terminals in the granular layer, the criteria for their identification being those given by BRODAL and GRANT (1962). It may be mentioned that degenerating mossy fibre terminals have the same appearance in the paramedian lobule and sublobule VIII B as in the anterior lobe vermis described by BRODAL and GRANT (1962).

Silver methods do not seem to have been used previously in studies of the cerebellar distribution of spinocerebellar tracts. The Marchi method and its modifications used by previous authors would not be expected to demonstrate unmyelinated or poorly myelinated fibres if such were present in the spinocerebellar tracts. In the present study the degenerating fibres to the paramedian lobule and the medialmost portion of the dorsal paraflocculus have been shown to have a smaller calibre than those to the other parts of the cerebellum, although there are scattered rather coarse fibres among them. Presumably these coarser fibres to the paramedian lobule are those which have been described by BECK (1927) in the cat and ANDERSON (1943) in the rat. They are said to be few, and other authors using the Marchi method have apparently not seen them. The silver methods used in the present study demon-

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1958). On an anatomical basis it must therefore be concluded that those cerebellar areas which receive fibres from the column of Clarke "represent" *the hindlimb and the trunk*.

From a functional point of view it is of interest to compare the cerebellar distribution of the dorsal spinocerebellar tract with that of the fibres from the external cuneate nucleus (see Fig. 15). This nucleus is supplied with dorsal spinal root afferents from cervical and upper thoracic segments (LIU 1956 and others) and is looked upon as the cervical cord equivalent to the column of Clarke (BRODAL 1941, JANSEN and BRODAL 1958 and others). The cerebellar projection of the external cuneate nucleus has been investigated in a separate study published elsewhere (GRANT 1962). It was demonstrated that the fibres from this nucleus like those of the dorsal spinocerebellar tract have two main terminal regions, an anterior and a posterior one. However, these two areas have an other location than those supplied by the dorsal spinocerebellar tract. The anterior area comprises lobule V, the anterior part of VI and possibly the posterior part of IV. The posterior area includes the pars posterior and the most anterior folium of the pars anterior² of the paramedian lobule as well as the folia in the depth of the fissura prepyramidalis, possibly comprising all folia of sublobule VIII A. Thus taking into account the origin of the spinal afferents to the external cuneate nucleus it may be concluded on an anatomical basis that the cerebellar areas receiving fibres from this nucleus "represent" *the neck, forelimb and uppermost part of the trunk*.

Turning now to the *ventral spinocerebellar tract*, this, in contrast to the dorsal tract, has a definite bilateral termination within the cerebellum. Furthermore, the contingent passing to the contralateral half of the cerebellum appears to be larger than the ipsilateral one.³ *It is of* interest that only very few fibres of the ventral spinocerebellar *tract* terminate in the midline region of the vermis proper in the anterior lobe, furthermore the maximal density of termination is found just lateral to a sagittal level passing *through the lateral extremity of lobule* I (see Figs. 12 and 14). Very few ventral spinocerebellar fibres seem to end in the paramedian lobule and in lobule VIII. A feature of special

² As has been mentioned the anterior folium of the pars anterior of the paramedian lobule appears to be supplied by afferent fibres *from* the dorsal spinocerebellar tract as well. These fibres, however, as compared with those from the external cuneate nucleus, seem to be restricted to the ventral portion of the folium.

³ The terms "ipsilateral" and "*contralateral*" when used here with regard to the ventral spinocerebellar tract, refer to the fibres ascending in the cord.

few fibres can be detected in sublobule VIII A. This is in accordance with the findings in the macaque by Voss (1952, 1953) and Vachananda (1959). The restriction of the degenerating fibres in the paramedian lobule to its posterior portion, the pars copularis, does not seem to have been noted by previous workers. Spinocerebellar fibres to the dorsal paraflocculus have been reported by Ingvar (1918). That no fibres have been seen to terminate in the cerebellar nuclei is in agreement with the findings of Beck (1927), Brodal and Jansen (1941), Whitlock (1952) and others. The fibres seen to pass through the fastigial nucleus are probably the same as those which were suggested by Ingvar (1918) to terminate in this nucleus.

Although the dorsal and ventral spinocerebellar tracts have in part identical areas of termination, there are minor but significant differences. The *dorsal spinocerebellar tract* terminates mainly, or perhaps exclusively, ipsilaterally in the anterior lobe and lobule VI, sublobule VII B, lobules VIII and IX, the paramedian lobule and the dorsal paraflocculus (Fig. 11). The degenerating fibres on the side contralateral to the lesion observed in cat K 68 and others seem to be restricted to those parts of the cerebellum which receive ventral spinocerebellar fibres. Probably this is due to the lesions encroaching slightly upon the dorsalmost fibres of the ventral tract in the spinal cord in these cases. This assumption is further strengthened by the fact that there are no degenerating fibres contralaterally in the cerebellum in cat K 175 in which the lesion is restricted to the dorsalmost portion of the dorsolateral quadrant and probably does not encroach upon ventral spinocerebellar fibres. No evidence has been found for a differential distribution of dorsal spinocerebellar fibres originating from different segments above the caudal end of the column of Clarke. It should be recalled, however, that dorsal root afferents to the column of Clarke overlap extensively (Liu 1956, Grant and Rexed 1958). Therefore, if there is a differential distribution of dorsal spinocerebellar fibres in the cerebellum this would be difficult to reveal with the approach used in the present study. Partial destruction of the column of Clarke or the application of the method of retrograde chromatolysis with restricted cerebellar lesions might possibly be more suited to solve this question.

The findings in cats K 99, 171 and 180 agree with the view that the origin of the dorsal spinocerebellar tract is the column of Clarke. This has been demonstrated to receive its dorsal spinal root afferents from the 3rd sacral up to the 2nd thoracic segment, while the more rostral segments do not give off afferents to the column (Grant and Rexed

material leaves no doubt that fibres of the ventral spinocerebellar tract originating at the lowest spinal levels terminate in the most anterior parts of the anterior lobe while fibres from more rostral segments reach more posterior regions (cp Figs 13 and 12)

Functional correlations

It is of considerable interest that the two cerebellar areas receiving spinocerebellar fibres correspond approximately with those demonstrated by neurophysiological methods to receive impulses from the hindlimb

ADRIAN (1943) has thus demonstrated that natural stimulation of various kinds of receptors (mixed stimulation of proprioceptors and exteroceptors) in the hindlimb in cats and monkeys may give rise to potentials in the lobulus centralis (Larsell's lobules II and III) of the anterior lobe Similar stimulation of the forelimb on the other hand may give rise to potentials restricted to the culmen (Larsell's lobules IV and V) SNIDER and STOWELL (1944) have recorded potentials from the anterior lobe as well as from the paramedian lobule following tactile stimulation of the hindpaw and forepaw in cats and monkeys Within the anterior lobe they found a hindpaw area within a region probably corresponding to the most anterior part of the culmen and the posterior part of the lobulus centralis (the most anterior parts of the anterior lobe were not investigated) Similarily they found a fore paw area in the posterior part of the culmen Within the paramedian lobule they found a hindpaw and a forepaw area somewhat over lapping each other As compared with the results of the present study it is of interest that their Fig 2 shows that the hindpaw area is situated in the pars copularis

COMBS (1954) using neurophysiological methods found evidence for a somatotopical localization in the anterior lobe in the cat A hindlimb region was found anterior to a forelimb region As compared with the results of the present study it is of interest furthermore that stimulation of the saphenous nerve gave rise to impulses restricted to an area extending anteriorly from exactly behind the anterior two folia of the culmen

Like the previous authors CARREA and GRUNDFEST [illegible] using cats [illegible] impulses [illegible] app[illegible] us that the [illegible] they recorded impulses following stimulation of the saphenous nerve extends posteriorly to the transition between Lar

interest is the demonstration of a segmental pattern of termination of the fibres in the anterior lobe. As regards the level of origin of the ventral spinocerebellar tract *in the spinal cord this is found in part at least caudal to that of the dorsal spinocerebellar tract. Degenerating fibres have been found in the cerebellum following a lesion as far caudally as in the 3rd sacral segment.*

The finding of a bilateral termination of the ventral spinocerebellar tract in the anterior lobe confirms earlier observations (Collier and Buzzard 1903, MacNalty and Horsley 1909, Beck 1927, Whitlock 1952 and others) made in birds and in mammals including cat and man. That most of the fibres terminate contralaterally is in agreement *with the findings of Auerbach (1890 b), Beck (1927)* and others. The paucity of ventral spinocerebellar fibres to the midline region of the vermis proper has apparently not been noted in previous anatomical *investigations. The fact that the ventral spinocerebellar tract terminates* medially in the anterior lobe (Fig. 12) is however in agreement with the observations of Beck (1927) and Anderson (1943). The findings in this study on the cat are not in agreement with regard to those reported by Chang and Ruch (1949) in the spider monkey that the fibres of the ventral spinocerebellar tract in the anterior lobe are confined strictly to the median plane. The divergent results might be explained on the basis of species variations. The paucity of fibres to the midline *region as well as a maximal density of termination just lateral to this* area seems to be in complete agreement with recent observations made by Lundberg and Oscarsson (1962) using neurophysiological methods. The observation that the ventral spinocerebellar tract in the cat terminates almost exclusively *within the anterior lobe is in agreement* with the findings of Ingvar (1918) and *Beck (1927). Fibres to the posterior* vermis have been described previously in the rat, spider monkey and the macaque (Anderson 1943, Chang and Ruch 1949, Yoss 1953 and *Vachananda 1959). In the cat these fibres seem to be very few as* those to the paramedian lobule.

A spinal origin of the ventral spinocerebellar tract below the caudal end of the column of Clarke has *been demonstrated in the monkey* (Bruce 1910, Yoss 1953, Vachananda 1959). Conditions have been assumed to be similar in the cat (Auerbach 1890 a). This is confirmed *by the findings in this study.* The demonstration in the present study of a segmental pattern of termination of the ventral spinocerebellar tract within the anterior lobe confirms and *extends the observation of* Chang and Ruch (1949) who suggested its existence. The present

material leaves no doubt that fibres of the ventral spinocerebellar tract originating at the lowest spinal levels terminate in the most anterior parts of the anterior lobe while fibres from more rostral segments reach more posterior regions (cp Figs 13 and 19)

Functional correlations

It is of considerable interest that the two cerebellar areas receiving spinocerebellar fibres correspond approximately with those demonstrated by neurophysiological methods to receive impulses from the hindlimb

ADRIAN (1943) has thus demonstrated that natural stimulation of various kinds of receptors (mixed stimulation of proprioceptors and exteroceptors) in the hindlimb in cats and monkeys may give rise to potentials in the lobulus centralis (Larsell's lobules II and III) of the anterior lobe Similar stimulation of the forelimb on the other hand may give rise to potentials restricted to the culmen (Larsell's lobules IV and V) SNIDER and STOWELL (1944) have recorded potentials from the anterior lobe as well as from the paramedian lobule following tactile stimulation of the hindpaw and forepaw *in cats and monkeys* Within the anterior lobe they found a hindpaw area within a region probably corresponding to the *most anterior* part of the culmen and the posterior part of the lobulus centralis (the most anterior *parts of* the anterior lobe were not investigated) Similarly they *found a fore*paw area in the posterior part of *the culmen* Within *the* paramedian lobule they found a hindpaw and a forepaw area somewhat

COMBS (1954) using neurophysiological methods found *evidence for* a somatotopical localization in the anterior lobe *in the cat* A hindlimb region was found anterior to a *forelimb region* As compared with the results of the present study it *is of interest* furthermore that stimulation of the saphenous *nerve gave rise* to impulses restricted to an area extending anteriorly *from exactly* behind the anterior two folia of the culmen

Like the previous authors LARREA and GRUNDFEST (1954) using cats and monkeys have demonstrated a somatotopical pattern for impulses recorded from the anterior *lobe* From their Fig 14 it appears th t the area within which they the
saphenous nerve extend Lar

interest is the demonstration of a segmental pattern of termination of the fibres in the anterior lobe. As regards the level of origin of the ventral spinocerebellar tract in the spinal cord this is found in part, at least, caudal to that of the dorsal spinocerebellar tract. Degenerating fibres have been found in the cerebellum following a lesion as far caudally as in the 3rd sacral segment.

The finding of a bilateral termination of the ventral spinocerebellar tract in the anterior lobe confirms earlier observations (COLLIER and BUZZARD 1903, MACNALTY and HORSLEY 1909, BECK 1927, WHITLOCK 1952, and others) made in birds and in mammals, including cat and man. That most of the fibres terminate contralaterally is in agreement with the findings of AUERBACH (1890 b), BECK (1927), and others. The paucity of ventral spinocerebellar fibres to the midline region of the vermis proper has apparently not been noted in previous anatomical investigations. The fact that the ventral spinocerebellar tract terminates medially in the anterior lobe (Fig. 12) is, however, in agreement with the observations of BECK (1927) and ANDERSON (1943). The findings in this study on the cat are not in agreement with regard to those reported by CHANG and RUCH (1949) in the spider monkey that the fibres of the ventral spinocerebellar tract in the anterior lobe are confined strictly to the median plane. The divergent results might be explained on the basis of species variations. The paucity of fibres to the midline region as well as a maximal density of termination just lateral to this area seems to be in complete agreement with recent observations made by LUNDBERG and OSCARSSON (1962) using neurophysiological methods. The observation that the ventral spinocerebellar tract in the cat terminates almost exclusively within the anterior lobe is in agreement with the findings of INGVAR (1918) and BECK (1927). Fibres to the posterior vermis have been described previously in the rat, spider monkey and the macaque (ANDERSON 1943, CHANG and RUCH 1949, YOSS 1953 and VACHANANDA 1959). In the cat these fibres seem to be very few, as those to the paramedian lobule.

A spinal origin of the ventral spinocerebellar tract below the caudal end of the column of Clarke has been demonstrated in the monkey (BRUCE 1910, YOSS 1953, VACHANANDA 1959). Conditions have been assumed to be similar in the cat (AUERBACH 1890 a). This is confirmed by the findings in this study. The demonstration in the present study of a segmental pattern of termination of the ventral spinocerebellar tract within the anterior lobe confirms and extends the observation of CHANG and RUCH (1949) who suggested its existence. The present

of this projection (POMPEIANO 1960) Furthermore the vermis of the anterior lobe seems to project in a somatotopical manner not only onto the nucleus of Deiters (WALBERG and JANSEN 1961) but also onto the fastigial nucleus (JANSEN and BRODAL 1940 1942) which again projects in a localized pattern onto the nucleus of Deiters (WALBERG POMPEIANO BRODAL and JANSEN 1962) This localization has been demonstrated in physiological studies as well (see BRODAL POMPEIANO and WALBERG 1962) Thus the cerebellum is interconnected with the spinal cord by efferent and afferent pathways which make possible an intimate correlation between them on a somatotopical basis The results of the present study are a contribution towards the solution of the larger problem of cerebellar localization

sell's lobules IV and V. Posterior to this they found potentials following stimulation of the superficial radial nerve.

MORIN and LINDNER (1953) investigating the paramedian lobule in cats reported that the potentials of maximal amplitude were found in the lowest two folia following light touch applied to the hindfoot. These folia belong to the pars copularis of the paramedian lobule.

DOW and ANDERSON (1942) have recorded potentials from the pyramis (Larsell's lobule VIII) following sudden taps of the tendons of the quadriceps and triceps muscles in rats. They did not, however, find support for a somatotopical localization of the potentials.

Turning now to a consideration of the spinocerebellar tracts there are physiological observations of interest for the present study.

GRUNDFEST and CAMPBELL (1942), working before the somatotopical pattern in the anterior lobe had been demonstrated, mentioned that cerebellar potentials due to direct activity of dorsal spinocerebellar tract fibres are largest in the lobulus centralis and the immediately adjacent folia of the culmen. Their Fig. 15 shows a posterior limit for the field within which these potentials were recorded corresponding rather exactly to the one for the termination of the dorsal spinocerebellar tract as demonstrated in the present study. As regards the ventral spinocerebellar tract it is of interest that HOLMQVIST and OSCARSSON (personal communication 1962) using physiological methods have not found a representation of the forelimb within it. This negative finding would be expected since the terminal area of the tract is restricted to the forelimb region of the anterior lobe.

The dorsal as well as the ventral spinocerebellar tracts transmit proprioceptive impulses to the cerebellum (GRUNDFEST and CAMPBELL 1942, LAPORTE, LUNDBERG and OSCARSSON 1956, OSCARSSON 1957). The route via the lateral reticular nucleus is the only one previously known to be anatomically organized in a manner that would permit transmission of somatotopically localized spinal impulses to the cerebellum (BRODAL 1941, 1949, see also JANSEN and BRODAL 1958). This system seems to transmit exteroceptive impulses (COMBS 1954, 1956, BOHM 1953). Therefore the present investigation brings the first anatomical evidence for a somatotopically localized termination of two spinal tracts transmitting proprioceptive impulses to the cerebellum.

It is of interest that recent studies indicate a localization in the projection of the cerebellum to the vestibular nuclei and that these project somatotopically to the spinal cord. The nucleus of Deiters has been shown to project in a somatotopical manner onto the cord (POMPEIANO and BRODAL 1957). Physiological studies have confirmed the existence

demonstrated The maximal density of termination in the anterior lobe was found just lateral to a sagittal level passing approximately through the lateral extremity of lobule I The midline region is very sparsely supplied by ventral spinocerebellar fibres

Most of the spinocerebellar fibres to the paramedian lobule and the dorsal paraflocculus seem to be of a fine calibre

Summary

In Chapter I is presented a study of ascending degeneration in the spinal cord following incisions made at different segmental levels and involving various parts of the lateral columns Ensuing degeneration was studied in transverse sections prepared according to the method of Swank and Davenport Three contingents of ascending degenerating fibres were identified Two of them were interpreted as belonging to the ventral and dorsal spinocerebellar tracts The third contingent most probably constitutes spinal afferents to the lateral cervical nucleus The ventral spinocerebellar tract was found to turn dorsally during its course through the thoracic and lower cervical cord In the upper cervical segments it returns to a more ventral position Findings were made which support the conception that the fibres composing the spinocerebellar tracts are topically arranged

In Chapter II is presented a study of the terminal distribution of the spinocerebellar tracts following incisions made at different levels of the cord involving more or less the ventral and/or the dorsal spino cerebellar tract Approximately serial sagittal sections through the cerebellum were treated according to the method of Nauta (using the Laud law solution) and/or that of Nauta and Gygax and some according to the methods of Glees and Reumont and Lhermitte The distribution of the degenerating fibres in the cerebellum was mapped The degeneration interpreted as a result of lesion of the dorsal spinocerebellar tract was confined to two areas ipsilaterally, one anteriorly and one posteriorly The anterior area comprises Larsell s lobules I to IV and the most anterior part of V The posterior area comprises sublobule VIII B the pars copularis of the paramedian lobule and the medialmost portion of the dorsal paraflocculus Since the column of Clarke receives dorsal spinal root afferents from at least the 3rd sacral up to the 2nd thoracic segment, these cerebellar areas "represent" the hindlimb and the trunk The terminal areas correspond rather well to the "hindlimb areas" determined by physiological methods

The ventral spinocerebellar tract was found to terminate within the same cerebellar areas as the dorsal tract but mainly contralaterally Its contribution to the posterior area seems to be very restricted A segmental pattern of termination of its fibres in the anterior lobe was

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Plates

E



C 2

D



C 6

C



B



T 12

A



L 1

Fig 1

A Diagram of a transverse section of the spinal cord at I 1 showing the extent of the lesion (hatched) x 6

B–L Transverse sections of the spinal cord at the indicated levels showing degenerating fibres (white) Swank and Davenport method x 10

R L

F



C1

E



C3

D



C8

C



T5

B



T13

A



L1

R 1

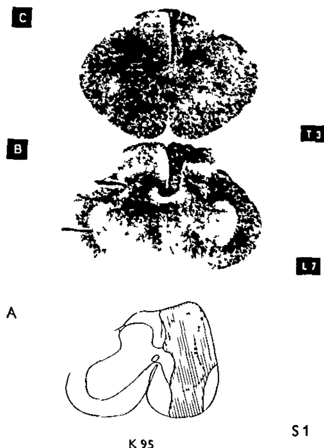


Fig 2

K 95

Figs 2 and 3

A Diagrams of transverse sections of the spinal cord at the indicated levels showing the extent of the lesions (hatched) x 6

B C and B L respectively Transverse sections of the spinal cord at the indicated levels showing degenerating fibres (white) Swank and Davenport method x 10

Arrow in Fig 3 L indicates an invagination of the surface of the lateral column

R L

F



E



D



C

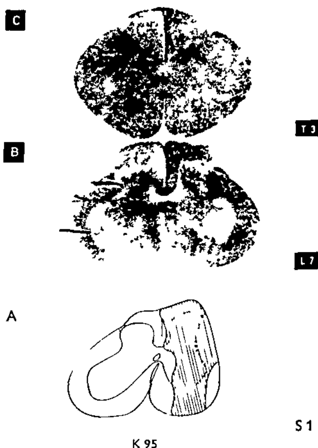


B



A





Figs. 2 and 3.

A. Diagrams of transverse sections of the spinal cord at the indicated levels showing the extent of the lesions (hatched). × 6.

B, C and B, I, respectively. Transverse sections of the spinal cord at the indicated levels showing degenerating fibres (white). Swank and Davenport method. × 10.

Arrow in Fig. 3 I indicates an invagination of the surface of the lateral column.

R L

D

T 1

C

T 6

B

T 10



A Diagram of a transverse section of the spinal cord at L 2 showing the extent of the lesion (hatched) x 6

B–D Transverse sections of the spinal cord at the indicated levels showing degenerating fibres (white) Swank and Davenport method x 10

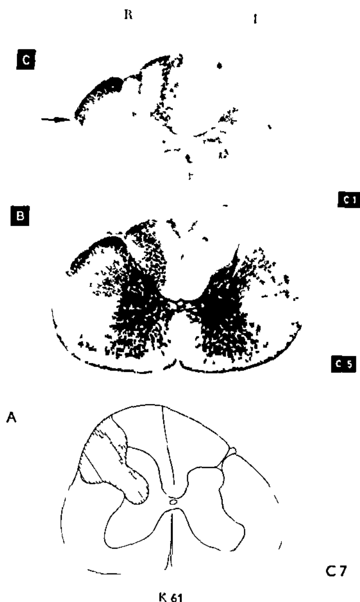


Fig 4

A Diagram of a transverse section of the spinal cord at C 7 showing the extent of the lesion (hatched) x 6

B–C Transverse sections of the spinal cord at the indicated levels showing degenerating fibres (white Swank and Davenport method x 10

Arrow in C indicates an invagination of the surface of the lateral column





Fig 7

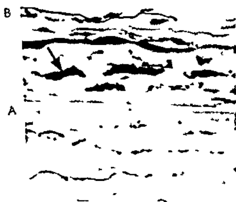


Fig 8

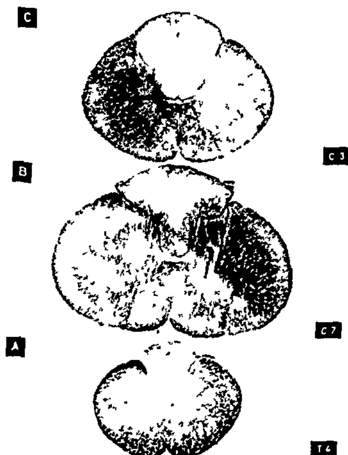


Fig 6

Fig 6

Transverse sections of the spinal cord (cat K 147) at the indicated levels showing ascending degeneration note the change in configuration of the degenerating field just lateral to the root entry zone *in the different sections Some pseudo degeneration* is visible along parts of the periphery of the sections in B and C Swank and *Davenport method* x 10

Fig 7

A Diagram of a transverse section of the spinal cord at T 6 showing the extent of the lesion (hatched) x 6

B Transverse section of the spinal cord at C 1 showing degenerating fibres white Swank and Davenport method x 10

Fig 8

Degenerating fibres in the white matter of the cerebellum (cat K 68) x 1300

A From the pars copularis of the paramedian lobule In the middle of the picture is a *thin degenerating fibre*

B From the anterior lobe A fragment of a coarse degenerating fibre is arrowed and above it a coarse non degenerating fibre

B



C1

A



K 84

T6

Fig 7

B



A

Fig 8

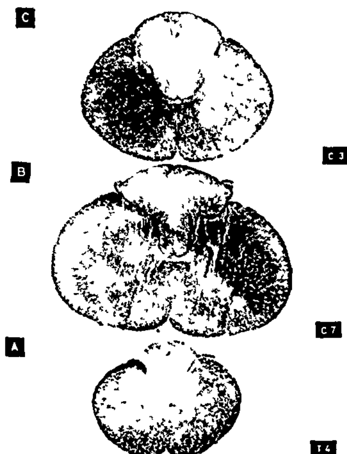


Fig 6

Fig 6

Transverse sections of the spinal cord (cat K 117) at the indicated levels showing ascending degeneration—note the change in configuration of the degenerating field just lateral to the root entry zone in the different sections (Some pseudodegeneration is visible along parts of the periphery of the sections in B and C) Swank and Davenport method x 10

Fig 7

A Diagram of a transverse section of the spinal cord at T 6 showing the extent of the lesion (hatched) x 6

B Transverse section of the spinal cord at C 1 showing degenerating fibres white Swank and Davenport method x 10

Fig 8

Degenerating fibres in the white matter of the cerebellum (cat K 68) x 1300

A From the pars copularis of the paramedian lobule In the middle of the picture is a thin degenerating fibre

B From the anterior lobe A fragment of a coarse degenerating fibre is arrowed and above it a coarse non-degenerating fibre

R L

C

N d N N t

pfl v

pfl d

Crus II

Crus I

I pmd copul

I pmd post

I pmd ant

IX

VIII B

VIII A

VII B

VII A

VI

V

IV

III

II

I

B

VI

f pr

VII A

A

VIII B

II

f prc

A

f apm

I pmd ant

I pmd post

I pmd copul

pf d

Fig. 10

K 153



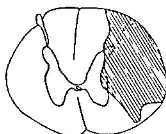


Fig. 9

Fig. 9.

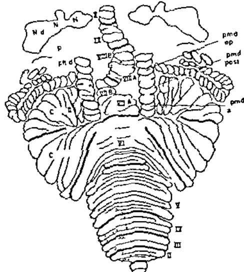
A. Diagram of a transverse section of the spinal cord at C 3 showing the extent of the lesion (hatched). × 6.
B. A transverse section at a caudal level of the medulla oblongata showing degenerating fibres (white). Swank and Davenport method. × 10.

Fig. 10.

C. Terminal regions of degenerating fibres (dotted) within the cerebellum (represented unfolded) following the lesion at C 3 shown in Fig. 9 A. The diagram of the cerebellum is modified from BRODAL (1941). The spacing of the dots serves to give an impression of the intensity of the degeneration in the various regions.
B. Diagram of a sagittal section through the cerebellum of cat K 153 somewhat to the left of the median plane showing the anteroposterior distribution of degeneration (indicated by interrupted lines) in the vermis.
A. Diagram of a sagittal section through the left paramedian lobule of the cerebellum of cat K 153 showing the anteroposterior distribution of degeneration (indicated by interrupted lines).

Abbreviations used in Fig. 10 and in Figs. 11, 12, 13 and 15: f. apm.: fissura ansoparamediana; f. ic. 1: fissura intracentralis 1; f. icul. 1: fissura intraculminata 1; f. ipd.: fissura intrapyramidalis; fl.: flocculus; f. pc.: fissura preculminata; f. ppd.: fissura prepyramidalis; f. pr.: fissura prima; f. prc.: fissura precentralis; f. sec.: fissura secunda; l. pmd. ant.: pars anterior lobuli paramediani; l. pmd. post.: pars posterior lobuli paramediani; l. pmd. copul.: pars copularis lobuli paramediani; N. d.: nucleus dentatus; N. f.: nucleus fastigii; N. i.: nucleus interpositus; pfl. d.: paraflocculus dorsalis; pfl. v.: paraflocculus ventralis; I, II, III, IV, V, VI, IX, X, VII A, VII B, VIII A, VIII B: cerebellar lobules and sublobules according to LARSELL's nomenclature (1953). The subdivision of lobule VIII into two sublobules has been made more recently (LARSELL, personal communication 1961).

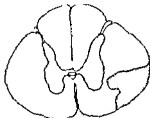
C





C1

A



C3

Fig. 1

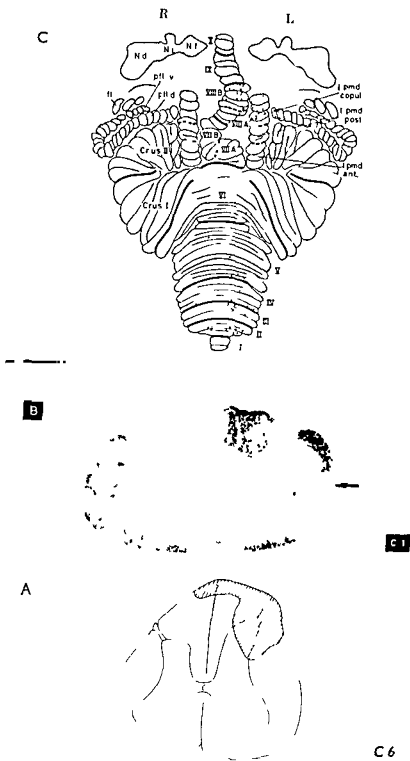

R
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fl
Pfl d
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Crus I
l pmd copul
l pmd post
l pmd ant.
B
C 1
A
C 6
K 68

Fig 11

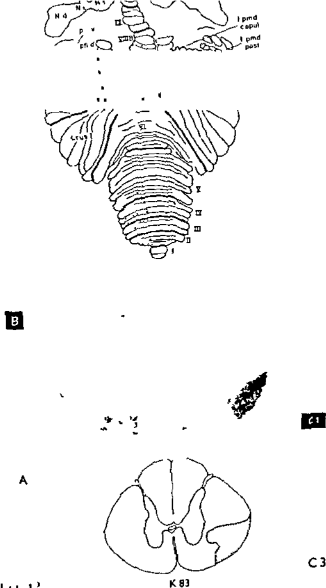

C
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VIIIB
l pmd
copul
l pmd
post
crus I
VI
V
IV
III
II
I
B
C1
A
K 83
C3

Fig 12

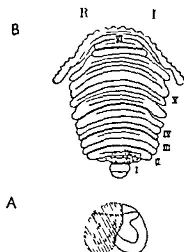


Fig 13 K 96



Fig 14 K 79

Fig 13

A Diagram of the spinal cord at S 3 showing the extent of the lesion (hatched) x C
B Diagram of the anterior part of the cerebellum showing the terminal region of degenerating fibres within the anterior lobe (dotted) following the lesion shown in A

Fig 14

Diagram of a transverse section from the anterior lobe showing degenerating fibres belonging to the ventral spinocerebellar tract following a lesion of this tract on the contralateral side of the spinal cord

Fig 15

C Diagram of the cerebellum (represented unfolded) showing the terminal regions of the spinocerebellar tracts (hatched regions) and of fibres from the external cuneate nucleus (black regions)
B and A Diagrams of sagittal sections through the vermis and the paramedian lobule respectively showing the anteroposterior distribution of the spinocerebellar tracts (hatched regions) and of fibres from the external cuneate nucleus (black regions)

C

B

A

Fig. 15

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CARL BLOMS BOKTRYCKERI A B

Chapter I

General introduction

The projection of the body to the cerebral cortex, as evidenced by the slow cortical potential evoked by electrical stimulation of nerves or by adequate stimulation of peripheral receptors is well known from a considerable number of investigations. The topographical pattern of cortical representation as well as the separation of the somatic sensory projection into two different areas the first somatic sensory area (S I) (MARSHALL, WOOLSEY and BARD 1937) and the second somatic sensory area (S II), were originally observed by ADRIAN (1941) and MARSHALL, WOOLSEY and BARD (1941) and have repeatedly been studied and confirmed (for references see ALBE FESSARD 1957, WOOLSEY 1958, AMASSIAN 1961, NAKAHAMA 1961).

The cortical projection of peripheral receptors has been further investigated by microelectrode recording from single cells. Particularly important findings have been made by MOUNTCASTLE and his associates. The majority of cells in S I are activated by gentle stimulation of skin or deep structures in small areas and are modality and place specific with regard to mechanical stimulation (MOUNTCASTLE 1957, TOWE and AMASSIAN 1958, MOUNTCASTLE and POWELL 1959 a, b). Activation of such units by changes in temperature has not been investigated in these studies. Such convergence can not be excluded since it has been demonstrated in cortical and thalamic cells activated from trigeminal innervation fields (LANDGREN 1957, 1961) and in cells in the dorsal horn of the spinal cord (WALL 1960).

Convergence has been observed between the somatic and visceral systems at thalamic (McLEOD 1958) and cortical (AMASSIAN 1951 a, b, 1952, NEWMAN 1962) levels. Convergence on single cells in the somatic sensory cortex has been found also between the specific and the non specific thalamic projection systems (LI 1956) and in addition S I and S II have been shown to have connections with several other cortical areas, both ipsilateral and contralateral (NAKAHAMA 1961). Finally, connections from the cerebellum to the somatic sensory cortex have been demonstrated (HENNEMAN, COOK and SNIDER 1952, COMBS and SAXON 1959).

Chapter I

General Introduction

The projection of the body to the cerebral cortex, as evidenced by the slow cortical potential evoked by electrical stimulation of nerves or by adequate stimulation of peripheral receptors, is well known from a considerable number of investigations. The topographical pattern of cortical representation as well as the separation of the somatic sensory projection into two different areas, the first somatic sensory area (S I) (MARSHALL, WOOLSEY and BARD 1937) and the second somatic sensory area (S II) were originally observed by ADRIAN (1941) and MARSHALL, WOOLSEY and BARD (1941) and have repeatedly been studied and confirmed (for references see ALBE-FESSARD 1957, WOOLSEY 1958, AMASSIAN 1961, NAKAHAMA 1961).

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Units of a type similar to those in S I were also common in S II (CARRERAS and LEVITT 1959 CARRERAS and ANDERSSON 1962) but in contrast to S I no cells in S II were driven by gentle movements of joints Although a few cells activated from large receptive fields and by strong stimuli have been observed in S I (MOUNTCASTLE and POWELL 1959 b) such cells were much more common in S II The receptive fields for these cells were sometimes discontinuous and changeable in size and the cells were not modality specific Interaction between different modalities is observed in the activity of single cells (CARRERAS and ANDERSSON 1962) as well as between the surface potentials evoked by somatic and auditory stimulation (BERMAN 1961 a b) These findings suggest differences in the projection to S I and S II This is also supported by electrophysiological studies of the thalamo cortical connections (KNIGHTON 1950 STRATFORD 1954) and by ablation experiments ROSE and WOOLSEY (1958) found degeneration in the ventral basal complex (nucl ventralis postero medialis and nucl ventralis postero lateralis) after ablation of S I but ablation of only S II gave small and inconstant degeneration MACCHI ANGELERI and GUAZZI (1959) report discrete degeneration in the ventral basal complex after lesions of S II

Microelectrode studies of the ventral basal complex have revealed cells with topographical organization and discharge properties corresponding to the cortical cells activated by gentle stimulation in small fields (GAZE and GORDON 1954 ROSE and MOUNTCASTLE 1959 POGGIO and MOUNTCASTLE 1960 LANDGREN 1961) These cells differ strikingly from those in the posterior group of thalamic nuclei[1] where the majority of cells are adequately activated by strong stimuli the fields are large and frequently discontinuous and interaction between different modalities is commonly observed (WHITLOCK and PERL 1959 KRUGER and ALBE FESSARD 1960 POGGIO and MOUNTCASTLE 1960 ALBE FESSARD and KRUGER 1962) These properties are similar to the cortical cells activated from large receptive fields or by strong stimuli

On the basis *of differences in peripheral receptive fields* modalities and discharge properties of single neurones ROSE and MOUNTCASTLE (1959) have suggested that the somatic afferent system should be divided into two functionally different divisions the *lemniscal system* and the *anterolateral* (spino thalamic) *system* which are assumed *to correspond to morpho*logically different ascending pathways (POGGIO and MOUNTCASTLE 1960)

[1] Defined as "The caudal portion of the dorsal thalamus of the cat that region behind the ventral and lateral nuclear masses" "*It includes the pulvinar lateral* posterior nucleus suprageniculate nucleus and parts of magnocellular division of medial geniculate body and posterior nucleus (POGGIO and MOUNTCASTLE 1960 p 2?)

The cells in the lemniscal system are activated by light stimuli in small receptive fields and show a high degree of topographical organization with modality and place specificity. The system is " tuned for action in rapid tempo and capable of presenting at the cortical level patterns of neural activity which are most sensitively determined by the temporal and spatial properties of the peripheral stimuli" (POGGIO and MOUNTCASTLE 1960 p 284). The cells in the anterolateral system are activated from extensive peripheral fields and frequently by stimuli destructive to tissue. The high susceptibility to anaesthesia the long latency of the thalamic and cortical responses and the sustained type of the discharge suggest that the anterolateral system mediates information different from that transmitted through the lemniscal system. It has been suggested that one function of the anterolateral system is to subserve the sensibility of pain (POGGIO and MOUNTCASTLE 1960).

Knowledge of the cortical projection of the body has been largely obtained in preparations with intact spinal cord although some investigations have been performed after selective lesions of ascending spinal pathways with recording evoked cortical slow waves in response to peripheral stimulation. The present study is undertaken in order to correlate the activation pattern of single cells in the somatic cortical area S II with activity in different spinal pathways. Functional connection of the dorsal columns with the somatic sensory cortex is well established (BOHM 1953 GARDNER and HADDAD 1953 MARK and STEINER 1958 NORRSELL and VOORHOEVE 1962). The pathway consists of three neurones. The first neurone has its cell body in the dorsal root ganglion and ascends in the dorsal column of the same side to relay in a dorsal column nucleus (nucl gracilis or nucl cuneatus). In cat only about 25 per cent of the myelinated fibres entering the dorsal column from the hindlimbs reach the dorsal column nuclei (GLEES and SOLER 1951). The second order neurone projects to the opposite thalamic ventral basal complex via the medial lemniscus (RANSON and INGRAM 1932 BOWSHER 1958 BUSCH 1961). The third neurone projects to the cortical sensory areas S I and S II.

MORIN (1955) showed in cat that a cortical response of short latency can be obtained in response to stimulation of the hindlimbs via a pathway in the lateral funiculus after section of the dorsal columns. He found that this pathway does not reach the dorsal column nuclei and assumed that it relayed in the nucl cervicalis lateralis (REXED and BRODAL 1951). The pathway crosses to the opposite ventral quadrant at the level of C1-2 and ascends in the medial lemniscus to the ventral basal complex of the thalamus (MORIN and THOMAS 1955 BUSCH 1961). A similar pathway exists from the forelimbs (CATALANO and LAMARCHE 1957 HOLMQVIST

and OSCARSSON 1962). The nucl. cervicalis lateralis has also a trigeminal component (WALL and TAUB 1962). LUNDBERG and OSCARSSON (1961) *identified an ascending tract in* the dorso-medial part of the lateral funiculus in cat and this tract has been shown to be the spinal part of MORIN's spino-cortical tract (NORRSELL and VOORHOEVE 1962). The first neurone *relays in the dorsal horn of the same side* from which it enters the cord (ECCLES, ECCLES and LUNDBERG 1960), the second in the nucl. cervicalis lateralis of the same side and the third in the ventral basal complex of the *opposite thalamus (VAN BEUSEKOM 1955, BUSCH 1961)*. In this paper this pathway shall be referred to as MORIN's *pathway* and its spinal component as the *lateral tract*.

Outside the dorsal columns and the lateral tract (heretofore referred to as the *specific projection pathways*), no short latency cortical slow wave potentials can be obtained in response to single electrical shocks or to adequate stimulation in cat (*MORIN 1955*, CATALANO and *LAMARCHE 1957*, MARK and STEINER 1958, NORRSELL and *VOORHOEVE 1962*).

Fibre systems ascending in the ventral spinal cord have been shown to reach the thalamic *level in the cat. There are histological evidences for a* direct projection to the ventral basal complex and to the intralaminar nuclei as well as to other diencephalic structures (GETZ 1952, ANDERSON *and BERRY 1959, MEHLER, FEFERMAN and NAUTA 1960, BUSCH 1961*). Electrophysiological experiments have also shown projection from antero-lateral pathways to these regions (GAZE and GORDON 1955, KRUGER and *ALBE-FESSARD 1960, POGGIO and MOUNTCASTLE 1960, PERL and WHIT*LOCK 1961, WHITLOCK and PERL 1961).

In the ventral cord quadrants of cat LUNDBERG and OSCARSSON (1962) *found fibres polysynaptically activated by flexion reflex afferents* (FRA: Group II and Group III muscle afferents, cutaneous and high threshold joint afferents) (ECCLES and LUNDBERG 1959) from wide receptive fields. *One type of these fibres, the so called bilateral ventral flexion reflex tract* (bVFRT), could be traced to the level of the inferior colliculi. Other fibres, comprising the so called contralateral ventral flexion reflex tract (cVFRT), have an unknown termination.

The present paper deals mainly with the cortical effects elicited via the dorsal columns and the lateral tract. Cortical activation obtained via pathways ascending in the ventral quadrants of the spinal cord has been described in a preliminary report (ANDERSSON 1962 b). Some further observations obtained in preparations with only the ventral quadrants intact will be given in chapter VII. Direct spino-cerebellar tracts will be considered in chapter VI.

Chapter II

Methods

a *Operation and anaesthesia* Successful experiments have been carried out in 60 adult cats Anaesthesia was induced with ether and during the first part of the operation ether was adminstered together with suitable amounts of air and oxygen At this stage the skin over the skull was opened the temporal muscle extirpated and the bone covering S II removed with a dental drill After laminectomy selective lesions were made in the spinal cord with the aid of fine forceps and a binocular dissecting microscope The types of lesions are described in the appropriate chapters Thereafter ether was discontinued and pentobarbitone sodium (Nembutal Abbott) was given intravenously During this stage the chamber was mounted (cf below) the dura covering S II was removed and all bleeding into the chamber carefully stopped Usually more than one hour elapsed between the discontinuation of the ether and the start of the recording All operative wounds were carefully closed and the estimated volume of blood lost was substituted with a Dextrane solution

During recording the anaesthesia was kept as light as possible without spontaneous movements of the animal To prevent reflex movements a neuromuscular blocking agent gallamine triethiodide (Flaxedil May & Baker Ltd) was given in many of the experiments The intermittent recovery from neuromuscular block permitted control of the anaesthetic state and additional pentobarbitone sodium was given usually in doses of 5—10 mg Another guide in judging the depth of the anaesthesia was the size of the pupils which was kept as small as possible When necessary the animal was kept on artificial respiration with a pump

The animal was warmed by a radiating lamp and the rectal temperature was maintained close to 38°C Particular attention was paid to keep the skin temperature normal (cf HUNT and MCINTYRE 1960)

b *Methods of recording* The microelectrodes used were glass pipettes filled with 4 molar NaCl Only electrodes with a very gradual taper were used The external [illegible]

[illegible]

The microscrew carrying the elec[illegible]
move freely on the top of a rigid ch[illegible]
the bone surrounding the opening [illegible]
pound (Dental Kerr) When the glass [illegible] with the microelectrode was put in position all air was expelled and the hydrodynamic relations in the intact head were at least to some extent re established In most experiments the movements of the cortex were negligible and it was often possible to observe the same unit

and Oscarsson 1962) The nucl cervicalis lateralis has also a trigeminal component (Wall and Taub 1962). Lundberg and Oscarsson (1961) identified an ascending tract in the dorso medial part of the lateral funiculus in cat and this tract has been shown to be the spinal part of Morin's spino cortical tract (Norrsell and Voorhoeve 1962) The first neurone relays *in the dorsal horn of the same side from which it enters the cord* (Eccles, Eccles and Lundberg 1960), the second in the nucl cervicalis lateralis of the same side and the third in the ventral basal complex of the opposite thalamus (van Beusekom 1955 Busch 1961) In this paper this pathway shall be referred to as Morin's *pathway* and its spinal component as the *lateral tract*

Outside the dorsal columns and the lateral tract (heretofore referred to as the *specific projection pathways*), no short latency cortical slow wave potentials can be obtained in response to single electrical shocks or to *adequate stimulation in cat (Morin 1955, Catalano and Lamarche 1957,* Mark and Steiner 1958, Norrsell and Voorhoeve 1962)

Fibre systems ascending in the ventral spinal cord have been shown to reach the thalamic level in the cat There are histological evidences for a direct projection to the ventral basal complex and to the intralaminar nuclei as well as to other diencephalic structures (Getz 1952, Anderson and Berry 1959, Mehler, Feferman and Nauta 1960 Busch 1961) Electrophysiological experiments have also shown projection from antero lateral pathways to these regions (Gaze and Gordon 1955 Kruger and Albe Fessard 1960, Poggio and Mountcastle 1960, Perl and Whitlock 1961 Whitlock and Perl 1961)

In the ventral cord quadrants of cat Lundberg and Oscarsson (1962) found fibres polysynaptically activated by flexion reflex afferents (FRA Group II and Group III muscle afferents, cutaneous and high threshold joint afferents) *(Eccles and Lundberg 1959) from wide receptive fields* One type of these fibres the so called bilateral ventral flexion reflex tract (bVFRT) could be traced to the level of the *inferior* colliculi Other fibres comprising the so called contralateral ventral flexion reflex tract (cVFRT) have an unknown termination

The present paper deals mainly with the cortical effects elicited via the dorsal columns and the lateral tract Cortical activation obtained via pathways ascending in the ventral quadrants of the spinal cord has been described in a preliminary report (Andersson 1962 b) Some further *observations obtained in preparations* with only the ventral quadrants intact will be given in chapter VII Direct spino cerebellar tracts will be considered in chapter VI

e *Anatomical studies* The lesions in the spinal cord were controlled histologically The part of the spinal cord containing the lesions was fixed in 10 % formalin, embedded in paraffin and sectioned at 50 μ. Every section was mounted for microscopic study Experiments with unsatisfactory lesions were excluded from the material

f *Nomenclature* Each unit has been labelled and units in the same experiment are given consecutive numbers The experiment was labelled according to the number in the series of a certain spinal lesion Experiments with only the dorsal columns intact are called DC and the units obtained referred to as DC units If only the dorsal part of the contralateral lateral funiculus was cut the symbol used is DCx In experiments with two complete hemisections in the upper cervical level the label L is used and experiments with transection of only the dorsal columns are indicated as Lx

Contralateral and *ipsilateral* refer to the side of cortical recording (left side in all cases) if not otherwise indicated

for hours To prevent breaking of the electrode and compression of the cortex the arachnoid membrane was opened with fine forceps in the place chosen for the penetration The electrode was observed through a microscope and oriented into the opening in the arachnoid The electrode could be moved vertically with an accuracy of 5 μ and the point of contact was established both visually and from the change of the noise level in the loudspeaker

The potential difference between the electrode tip and a second lead attached to the scalp was led through a cathode follower into a R C preamplifier (Grass P5) and the potential changes were displayed on a cathode ray oscilloscope (Tektronix 502) from which photographic records could be made with a kymograph camera (Grass C4D) The frequency response curve of the amplifier with open filters has its $^1/_2$ amplitude response between 0 1 Hz and 26 kHz Different high and low pass band filters were available

As soon as the dura was opened a photograph of the exposed cortex was taken through the dissecting microscope This photograph was immediately developed and on this the location of the individual penetrations was indicated The peripheral receptive fields of the units isolated were indicated on simple outline drawings of a cat Latency measurements were always made from at least 10 but usually 15—20 individual records

c *Methods of stimulation* In order to facilitate the location of small fields the hair was cut In all cells studied the entire surface of the animal was stimulated in order to find the receptive field If the discharge of the unit could be influenced the modality and the receptive field were established as carefully as possible Light mechanical stimulation of the skin was carried out with jets of air, a fine hair brush or a small blunt probe The elevated skin or exposed subcutaneous structures were stimulated with strong stimuli such as pressure or pinching with toothed forceps Joints were moved manually Vibratory stimulation was carried out with a tuning fork Exact timing of the stimulation was obtained with electrical pulses from a stimulator (Grass S4) delivered with a pair of needles insulated except for the tips This electrode permitted stimulation either in the skin or in deeper structures The duration of the electrical stimulus was usually 0 1 msec Timed tactile stimuli could also be given with a soft brush moved by an electromagnetic device

d *Identification of the action potentials of single neurones* The electrical signs of the action potentials of single cortical neurones have been described in several papers and recently reviewed by AMASSIAN (1961) It is generally assumed that initially negative spike potentials are electrical signs of a cell discharging at some distance from the tip of the recording electrode and most of the units studied have been of this type Constant polarity, stable height and the all or none response of the potentials have been the criteria used for single neurone identification When the electrode is further advanced the spike potential sometimes is inverted to an initially positive potential and the discharge may reflect signs of damage Positive potential cells with signs of damage have not been included in the material

In most records the activity of only one cell was studied but in a few instances two or three cells could be clearly differentiated and studied at the same time

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Chapter III

The dorsal column

Introduction

The primary afferents ascend ipsilaterally (with regard to the entrance into the dorsal column) and relay in the nucl gracilis and nucl. cuneatus (RAMÓN y CAJAL 1952, FERRARO and BARRERA 1935, GLEES, LIVINGSTON and SOLER 1951). The connection with the second order neurone has a high synaptic security (THERMAN 1941). KUHN (1949) demonstrated projection from skin to the dorsal column nuclei JOHNSON (1952) showed that single cells in these nuclei in the cat could be activated by movement of hair or by pressure. AMASSIAN and DE VITO (1957) confirmed this finding for cells in the nucl. cuneatus and demonstrated inhibitory and facilitatory effects. An extensive investigation of the peripheral receptive fields and discharge properties of the cells in nucl. gracilis has been carried out by GORDON and PAINE (1960) and GORDON and SEED (1961). The majority of cells were activated by afferents from the skin and only 10 % of the cells responded to movements of joints or to deep pressure. The receptive areas of the cells differed with the rostro-caudal location of the electrode in the nucleus. In particular, the cells in the rostral part of nucl. gracilis had large receptive fields, sometimes including all the hindbody ipsilateral to the nucleus whereas in the middle part of the nucleus 96 % of the cells had receptive fields confined to only the hindfoot and of these cells 35 % had peripheral fields of 1 cm^2 or less. The cells in the caudal part of nucl. gracilis had smaller fields than cells in the anterior part but larger than cells in the middle region. Afferent inhibition was obtained only in cells with small receptive fields. Spatial facilitation with shortening of latency was observed but was not related to the size of the receptive field. WALL (1961) confirmed that the projection of afferents to the dorsal column nuclei is mostly from the skin but also from joints. He did not find inhibitory effects from light stimuli. Similarly, inhibition could not be demonstrated by KRUGER, SIMINOFF and WITKOVSKY (1961), whose results also show other differences from those of GORDON and PAINE (1960).

Results

A. *Material and classification*

The present investigation of the projection to S II through the dorsal column pathway consists of two series of experiments with different spinal lesions. In the larger series (9 cats) only the dorsal columns were intact and the lateral and ventral spinal cord was sectioned bilaterally at the level of C_{3-4}. 199 units (DC units) were studied in 31 penetrations. In another series (4 cats) only the dorsal part of the contralateral lateral funiculus of the spinal cord was sectioned at the level of C_{3-4}. 86 units (DCv units) were studied in 16 penetrations. The extent of the lesions in each of the two series is shown in Fig. 1. The location of the penetrations is given in Fig. 23 B. Units were isolated at all depths of the cortex but the majority of the cells were found at the depth of 400 μ—1800 μ below the surface. Units below 2000 μ were found only occasionally. The distribution in depth of the cells isolated is shown in Fig. 2. Units activated by different modalities or with different sizes of peripheral receptive field were not confined to any particular depth in the cortex. Units with the same modality were usually obtained throughout the same penetration and some

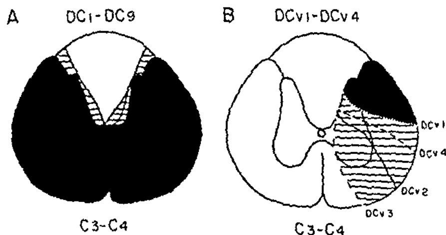


Fig. 1. Extent of spinal cord lesions. Solid black areas indicate parts of the spinal cord transected in all experiments, lined areas, the variation of the lesions between different experiments. (This convention shall hold for all spinal lesions pictured in this paper. A. Experiments with only the dorsal columns intact. B. Experiments with section of the dorsal part of the contralateral lateral funiculus. The extent of the lesion is shown for each experiment. In all experiments the lesions follow the pial septum between the dorsal column and the dorsomedial part of the lateral funiculus.

Chapter III

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Introduction

The primary afferents ascend ipsilaterally (with regard to the entrance into the dorsal column) and relay in the nucl. gracilis and nucl. cuneatus (Ramon y Cajal 1952, Ferraro and Barrera 1935, Glees, Livingston and Soler 1951). The connection with the second order neurone has a high synaptic security (Therman 1941). Kuhn (1919) demonstrated projection from skin to the dorsal column nuclei. Johnson (1952) showed that single cells in these nuclei in the cat could be activated by movement of hair or by pressure. Amassian and De Vito (1957) confirmed this finding for cells in the nucl. cuneatus and demonstrated inhibitory and facilitatory effects. An extensive investigation of the peripheral receptive fields and discharge properties of the cells in nucl. gracilis has been carried out by Gordon and Paine (1960) and Gordon and Seed (1961). The majority of cells were activated by afferents from the skin and only 10 % of the cells responded to movements of joints or to deep pressure. The receptive areas of the cells differed with the rostro-caudal location of the electrode in the nucleus. In particular, the cells in the rostral part of nucl. gracilis *had large receptive fields, sometimes including all the hindbody ipsilateral* to the nucleus, whereas in the middle part of the nucleus 96 % of the cells had receptive fields confined to only the hindfoot and of these cells 35 % had peripheral fields of 1 cm² or less. The cells in the caudal part of nucl. gracilis had smaller fields than cells in the anterior part but larger than cells in the middle region. Afferent inhibition was obtained only in cells with small receptive fields. Spatial facilitation with shortening of latency was observed but was not related to the size of the receptive field. Wall *(1961)* confirmed that the projection of afferents to the dorsal column nuclei is mostly from the skin but also from joints. He did not find inhibitory effects from light stimuli. Similarly, inhibition could not be demonstrated by Kruger, Siminoff and Witkovsky (1961), whose results also show other differences from those of Gordon and Paine (1960).

Results

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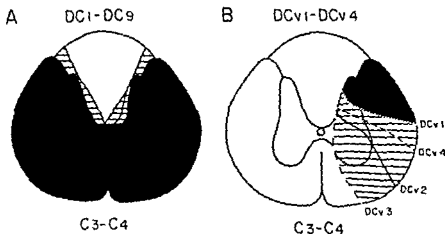


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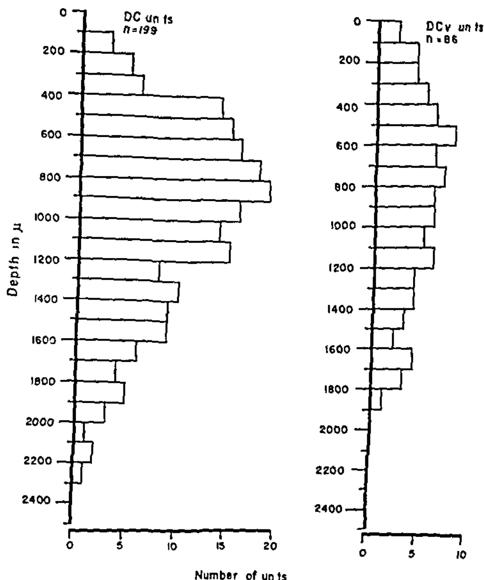


Fig 2 Depth distribution of cortical units studied in preparations with only the dorsal columns intact (left diagram) and in preparations with section of the dorsal part of the lateral funiculus (right diagram) Depth below the pial surface was determined by microscrew readings

times all the units had very similar fields and were activated by the same kind of stimulation Units activated by different modalities were rarely *intermingled in the same penetration but units activated from deep* structures and from skin were sometimes found in different parts of one penetration Cells driven by light touch from a small area were very seldom found close to units activated from large fields in the same penetration However in penetrations with units activated from large peripheral fields

Table 1

Modality location and extent of the receptive fields for all units (285) studied in DC and DCv experiments

Receptive fields		Modality	DC units	DCv units	Totals
Small	contralateral	Skin	154*	42	196
		Deep	9	17	26
Large	contralateral	Skin	15	11	26
		Deep	10	6	16
	bilateral	Skin	8	7	15
		Deep	0	0	0
	ipsilateral	Skin	3	3	6
		Deep	0	0	0
		Totals	199	86	285

* 3 of these units were only inhibited

by only light stimuli units activated by both light and strong stimuli were observed several times The large number of penetrations with cells of the same modality support the finding of a vertical organization in the cortex (MOUNTCASTLE 1957, POWELL and MOUNTCASTLE 1959) Isolation of single units was more difficult in the hindlimb area than in the fore limb area On the average, twice as many units were isolated per penetration in the forelimb area than in the hindlimb area in the same experiment

The units studied in the two series of experiments have been classified with regard to size and location of the receptive field (Table 1) The units have also been divided into "skin" and "deep" according to modality Units activated from both skin and deep structures are classified under "skin" The largest group consists of cells with small receptive fields activated from skin but a considerable number of units were driven by stimulation in large skin areas and by stimulation of deep receptors especially in the preparations with section of only the dorsal part of the contralateral lateral funiculus

B Units activated from small receptive fields

These units are described separately according to the modalities "skin" and "deep"

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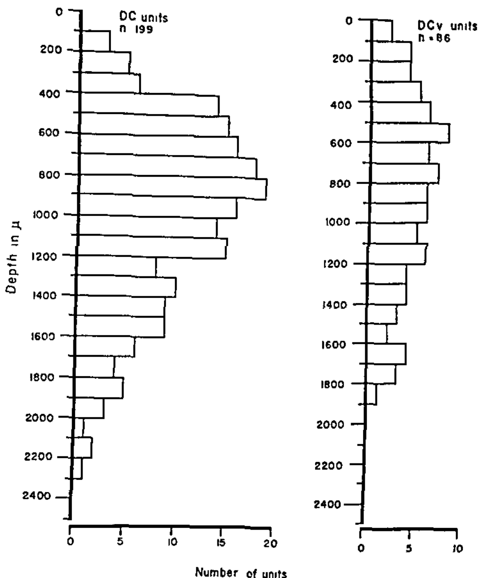


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B *Units activated from small receptive fields*

These units are described separately according to the modalities "skin" and "deep"

a *Units activated from small skin areas* 196 units with small cutaneous fields were activated exclusively from the contralateral side and most frequently from the distal parts of the limbs Such units constitute the

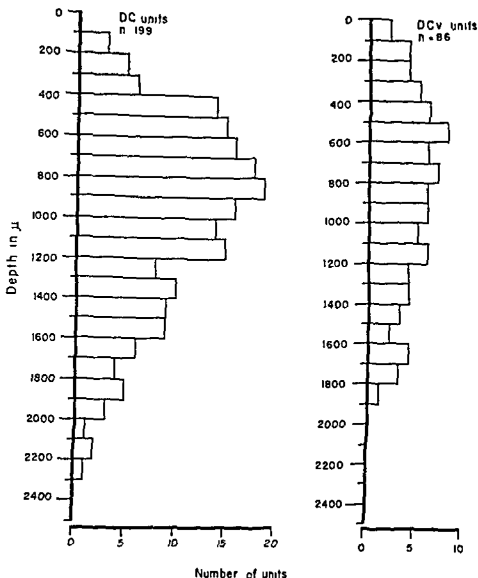


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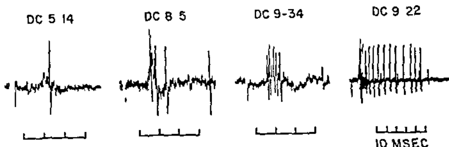


Fig 4 Different types of single cortical unit discharges evoked by high intensity electrical stimulation in the receptive fields of units adequately activated by light stimuli in small fields in preparations with only the dorsal columns intact Receptive field of unit DC 5—14 — pads contralateral hindfoot unit DC 8—5 — hairy skin contralateral forefoot unit DC 9—34 — hairy skin and pads contralateral forefoot unit DC 9—22 — hairy skin contralateral forefoot 10 msec between each vertical line of the time markers

the limb or of an ovoid shape (Fig 3 K—O) Many of these fields included the medial or the lateral part of the paw Some of the fields surrounded the limb like a cuff or a stocking but stimulation of one part of the field was usually much more effective than stimulation elsewhere in the field No differences in discharge properties were found between the cells with this type of receptive field and those with more restricted fields and they have been included in the group with small fields

The receptive fields of units activated by light stimulation in small fields of the skin was continuous The only exception was units activated from only pads The peripheral field was always constant during the time of observation although different depths of anaesthesia could affect the rate of spontaneous activity and the number of spikes in response to a stimulus Another invariable property was the modality in no case could these cells be affected by stimulation of deep structures The number of spikes varied between different units Most units discharged with only a few action potentials but some units discharged with a train of spikes (Fig 4)

The adaptation to a steady stimulus was usually fast with a few spikes at the onset of the stimulus A few units responded also at termination of the stimulus The slowly adapting units decreased their discharge rate during the first part of the stimulation and discharged thereafter at a more constant level At termination of the stimulus there was an abrupt cessation of the discharge usually followed by a decreased spontaneous rate for a few seconds The frequency of the spontaneous rate varied among different units Most of the fast adapting units had a low spontaneous rate or

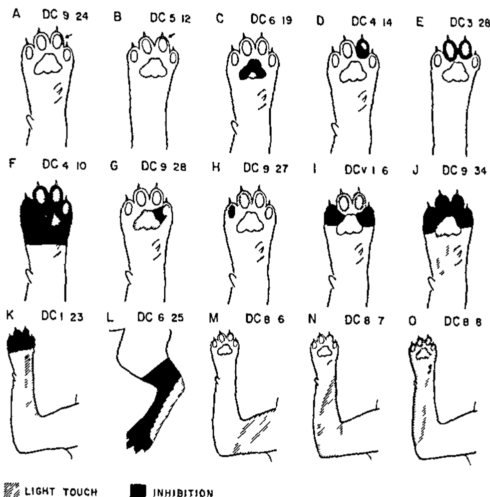


Fig 3 Location and size of typical excitatory and inhibitory fields of units activated by light stimuli to the skin of contralateral limbs in preparations with intact dorsal columns B L and L represent hindlimbs all the others forelimbs In A and B arrows indicate location of small fields Note inhibition in A

largest group in both series of experiments They were adequately activated by light stimuli such as jets of air bending of hairs or light pressure Many cells had an excitatory receptive area of less than 0 5 cm² on hairy skin or on a pad (Fig 3 A—D) Other cells were activated from more than one pad or hair on more than one toe (Fig 3 F—G) or from both hair and pads (Fig 3 I and J) The entire area of the paws was covered with overlapping small fields The average size of the fields on the fore paw was smaller than on the hindpaw where few very small fields were found and most fields covered several toes or pads

Fields were also found on more proximal parts of the limbs These fields were either long and narrow on either the preaxial or postaxial aspect of

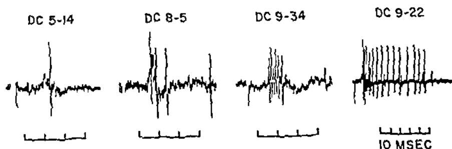


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discharged only sporadically when not stimulated whereas units with a slowly adapting response usually had a higher and continuous resting activity.

b. *Units activated by deep structures in small areas.* 26 units activated from deep receptors had small receptive fields. 10 units (7 DC units) had a low threshold and were activated by light stimulation. Thus, it was in every instance necessary to try to locate the receptors to be certain that the unit should be classified as deep rather than as cutaneous. Other units were activated from deep within the paws and these cells could usually be stimulated by pressure against the pads. 7 units (4 DC units) were activated from the region of a joint by strong stimuli. No unit responded to gentle movements of a joint. The activating stimulus was pressure on the joint capsule, hyperextension of the joint or strong movements.

All units with high and some with low thresholds to mechanical stimulation were slowly adapting to a steady stimulus.

c. *Afferent inhibition in units activated from small receptive fields.* In units activated from small skin fields the spontaneous rate of discharge or the response evoked by afferent stimulation could frequently be inhibited. This was found in both series of experiments with the dorsal columns intact. Table 2 shows the occurrence of inhibition in relation to the type of excitatory receptive field. In addition 3 units were found which only were inhibited from the distal part of a limb. Both the excitatory and the inhibitory effects were obtained from the skin and usually with the same type of stimulation. Table 2 shows that inhibition of the discharge from units activated from small fields was found in 35 % of the total population of such cells. Afferent inhibition was particularly com-

Table 2

Presence or absence of inhibition for all units studied in DC and DCv experiments

Type of fields	Inhibition *Nr of units*	No inhibition *Nr of units*	Totals
Only pads	41	28	69
Hair and pads	9	35	44
Hair, small fields	18	65	83
Large fields	2	44	46
Deep	2	38	40
Totals	72	210	282

3 units showing only inhibition are not included in this table

mon in cells activated from receptive fields on only pad 59 % of such cells could be inhibited In some experiments inhibition was more frequently obtained than in others The explanation might be due to differences in anaesthesia since cortical inhibition has high susceptibility to anaesthesia (MOUNTCASTLE and POWELL 1959 b) The finding that cortical inhibition is affected by anaesthesia was also observed in these experiments although not systematically studied Another difficulty in studying the inhibitory effects is the low spontaneous discharge in units activated from small fields Many of these cells discharge only at application of a stimulus Since inhibition can be studied only against a background activity brief tactile or electrical stimuli were employed to elicit the activity used as background in the search for inhibition when the cell did not discharge spontaneously

In units excited from small fields by light tactile stimuli the inhibitory field was continuous with the excitatory one and was often of the surrounding type Small excitatory fields on pads or hairy skin might be partially or completely surrounded by the inhibitory field (Fig 3 A C and D) In other units a larger excitatory field on several pads was interrupted by the inhibitory field (Fig 3 E—G) or the excitatory effect was inhibited from neighboring toes Fig 3 H and I) Units activated from hair or from hair and pads were found which could be inhibited only from pads (Fig 3 H) Units excited from long narrow fields were usually inhibited from a field distally on the same limb (Fig 3 J and K) Inhibitory effects on units with their receptive fields on the hindlimb were not as common as for cells driven from the forelimb The inhibitory fields on the hindlimb were usually larger than on the forelimb as were the excitatory fields themselves Only very few units showed the type of inhibition of a narrow field surrounding the excitatory area Inhibition from a larger area surrounding the excitatory fields (Fig 3 L) or distal to it were the most common type Inhibition of units driven by deep receptors was seen in 2 cases These units were activated from the region of a joint and inhibited by light touch to the paw of the same limb

d *Latency measurements* The latency of the cortical response (stimulus to first spike) to a brief electrical stimulus applied to the skin was measured in 190 units activated by light tactile stimulation from small fields in the contralateral forepaw or hindpaw The distribution of the shortest latency found in each of these units is shown in Fig 5 Inset in the figure are histograms showing the range of latency for units with different types of small receptive fields There is no significant difference

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b *Units activated by deep structures in small areas* 26 units activated from deep receptors had small receptive fields 10 units (7 DC units) had a low threshold and were activated by light stimulation Thus it was in every instance necessary to try to locate the receptors to be certain that the unit should be classified as deep rather than as cutaneous Other units were activated from deep within the paws and these cells could usually be stimulated by pressure against the pads 7 units (4 DC units) were activated from the region of a joint by strong stimuli No unit responded to gentle movements of a joint The activating stimulus was pressure on the joint capsule, hyperextension of the joint or strong movements

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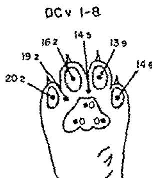


Fig 6 The mean latency in msec to high intensity electrical stimulation at different points in the receptive field of a unit adequately activated by light stimuli in the contralateral forefoot in a preparation with intact dorsal columns Note that there is no response from the central pad

in latency to stimulation of the forefoot between the subgroups but the latency of units activated from the hindfoot is shortest for the units activated from fields on hairy skin The latency was longer in units which showed afferent inhibition The average latency of 54 units activated from receptive fields on the contralateral forefoot and in which inhibition could not be found was 11 1 msec compared with an average of 13 6 msec for 2[illegible] units with inhibition and similar location of the receptive field The average latency of all units with small receptive fields was 12 1 msec for the forefoot and 19 4 msec for the hindfoot

The latency of a particular unit was not constant within its receptive field In parts of the field with less intense response to adequate stimulation the latency was longer The increase in latency was most pronounced at the edges of the field but the shortest latency was not always found in its geometrical center Fig 6 shows latencies obtained with electrical stimulation of the same intensity at several points in a field of a cell adequately activated by light touch The shortest latency was 13 9 msec and the longest 20 2 msec In most cells the latency was related to the intensity of the stimulus Latency studies with increasing intensity of stimulation from the threshold value or values close to threshold were made on 20 cells In Table 3 the latency at threshold and at maximum stimulation intensity are given together with the value of maximal stimulus expressed as multiples of the threshold intensity The increase in latency from maximum intensity of the stimulus down to lowest intensity used is given in msec and in percentage of the shortest latency The most rapid change in latency occurred usually close to the threshold value of

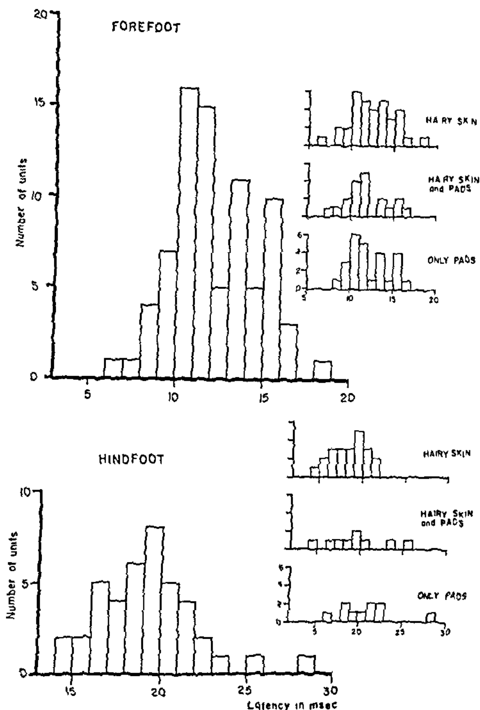


Fig 5 Distribution of the mean latencies for units activated by high intensity electrical stimuli from small receptive fields in the contralateral forefoot (79 units) and hindfoot (41 units) in preparations with intact dorsal columns. Inset histograms show the distribution of the mean latencies for units with different types of small peripheral receptive fields. Note that the latency axes begin at different values

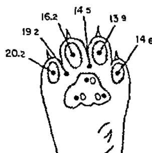


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Table 3

Latency changes to changes in the intensity of an electrical stimulus applied in the receptive field for 20 units adequately activated by light stimuli in small fields on contralateral fore (F) or hindfoot (H)

Unit	Latency, Threshold Stimulus	Latency Maximum Stimulus	Stimulus × Threshold	Latency Change in msec	and %
DCs 3— 6 F	24 7	10 5	100 0	14 2	135
DCs 1—14 I	24 6	11 9	8 8	12 7	107
DC 9—32 F	18 9	10 4	43 0	8 5	82
DCs 4—14 I	17 5	10 7	50 0	6 8	64
DCs 3—22 F	10 7	7 1	2 8	3 6	51
DC 9—28 I	17 2	11 8	43 0	5 4	46
DC 5— 4 H	23 8	16 8	10 0	7 0	42
DC 8— 5 F	15 4	11 0	75 0	4 4	40
DC 4—21 I	16 0	11 8	37 0	4 2	36
DC 4—22 F	15 6	11 5	23 0	4 1	36
DC 7—15 I	18 2	14 2	37 5	4 0	28
DC 9—34 F	12 9	10 4	30 0	2 5	24
DC 6— 5 F	13 1	10 8	50 0	2 3	21
DC 9—25 I	11 0	9 2	14 3	1 8	20
DC 4—28 I	15 8	13 4	3 7	2 4	18
DC 3—10 H	17 3	16 3	4 3	1 0	6
DC 3— 7 F	15 1	14 4	25 0	0 7	5
DC 6— 4 F	10 3	10 1	6 5	0 2	2
DC 3— 9 H	16 0	16 0	10 0	0 0	0
DC 7— 7 F	9 8	11 2	50 0	—1 4	—14

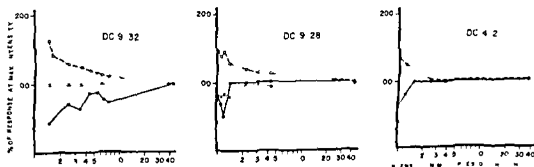


Fig 7 Graphs of data obtained in intensity series carried out for 3 units in preparations with only the dorsal columns intact Varied intensity electrical stimulation In units DC 9—32 and DC 9—28 afferent inhibition could be demonstrated In unit DC 4—21 no inhibition was found Open circles and dashed lines — mean latencies solid circles and solid lines — mean number of impulses per response crosses and dotted lines — probability that the response will occur Each point is the average of 10—20 single records

the stimulus (Fig 7) No decrease or lengthening in latency was found in 2 cells and in 3 other cells the decrease was very small

All the units with lengthening of the latency with increasing intensity of stimulation could also be inhibited by adequate stimulation in an area close to the excitatory field In a few units with afferent inhibition response was obtained only by weak electrical shocks and an increased intensity abolished the response A few units showed an increased latency and/or decreased number of spikes in the response when the intensity was raised to a certain value but a decrease in latency and/or increase in the number of spikes to higher intensities (Fig 7 units DC 9—32 and DC 9—28) Units without inhibition showed more regular curves (Fig 7 unit DC 4—21) Measurement of the latency of the cortical response was made in 15 units activated from deep structures The latencies obtained varied widely in different units 8 5—21 6 msec (mean 13 5 msec) in the forelimb and 10 ?—33 msec (mean 19 6 msec) in the hindlimb

C Units activated from large receptive fields

a *Fields and modality and discharge properties* In this group 47 units (26 DC units) could be activated from extensive skin areas sometimes including the entire animal except the head The fields frequently covered stocking like areas both on forelimb and hindlimb and some of the bilateral fields covered symmetrical parts of the body (Fig 8)

There was a different distribution of these units in the two series of experiments in preparations with section of only the dorsal part of the contralateral lateral funiculus units with large fields were about twice as common as in preparations with only the dorsal columns intact

The large fields were either continuous or discontinuous Most of the units with contralateral large fields had similar activation patterns as units with small fields The size of the receptive field was the most significant difference (compare Figs 3 and 6) They were place specific and activated only by light tactile stimulation 8 units (5 DC units) were not

[illegible] light tactile stimulation in one area and influenced only by strong stimulation such as pinching or pressure in other areas Fig 8 F and G) The units activated by both light and strong stimulation of the skin did not have constant receptive fields Slightly increased depth of anaesthesia abolished the effect of strong stimuli while the response to tactile stimulation remained practically unchanged Sometimes repeated manipulation of the animal could increase the receptive fields of such

Table 3

Latency changes to changes in the intensity of an electrical stimulus applied in the receptive field for 20 units adequately activated by light stimuli in small fields on contralateral fore (F) or hindfoot (H)

Unit	Latency, Threshold Stimulus	Latency Maximum Stimulus	Stimulus × Threshold	Latency Change in msec and %	
DCx 3—6 F	24.7	10.5	100.0	14.2	135
DCx 1—14 F	24.6	11.9	8.8	12.7	107
DC 9—32 F	18.9	10.4	43.0	8.5	82
DCx 4—14 F	17.5	10.7	50.0	6.8	64
DCx 3—22 F	10.7	7.1	2.8	3.6	51
DC 9—28 F	17.2	11.8	43.0	5.4	[illegible]
DC 5—4 H	23.8	16.8	10.0	7.0	42
DC 8—5 F	15.4	11.0	75.0	4.4	40
DC 4—21 F	16.0	11.8	37.0	4.2	36
DC 4—22 F	15.6	11.5	23.0	4.1	36
DC 7—15 F	18.2	14.2	37.5	4.0	28
DC 9—34 F	12.9	10.4	30.0	2.5	24
DC 6—5 F	13.1	10.8	50.0	2.3	21
DC 9—25 F	11.0	9.2	14.3	1.8	20
DC 4—28 F	15.8	13.4	3.7	2.4	18
DC 3—10 H	17.3	16.3	4.3	1.0	6
DC 3—7 F	15.1	14.4	25.0	0.7	5
DC 6—4 F	10.3	10.1	6.5	0.2	2
DC 3—9 H	16.0	16.0	10.0	0.0	0
DC 7—7 F	9.8	11.2	50.0	−1.4	−14

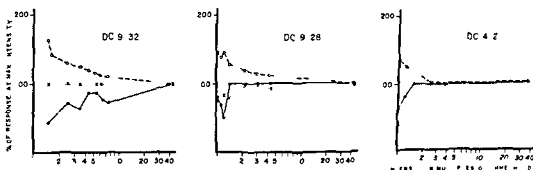


Fig. 7 Graphs of data obtained in intensity series carried out for 3 units in preparations with only the dorsal columns intact. Varied intensity electrical stimulation. In units DC 9—32 and DC 9—28 afferent inhibition could be demonstrated. In unit DC 4—21 no inhibition was found. Open circles and dashed lines — *mean latencies*, solid circles and solid lines — mean number of impulses per response, crosses and dotted lines — probability that the response will occur. Each point is the average of 10—20 single records.

b. *Afferent inhibition.* Inhibitory effects from somatic stimulation were systematically looked for but could be demonstrated only in two units (Table 2). Both were activated by light stimuli in large unilateral fields and were inhibited by similar stimuli in a large area on the opposite side. The fields of one of these units are shown in Fig. 8 H.

c. *Latency measurements.* The latency of the cortical response to a short electrical stimulus could frequently be measured in units adequately activated by light stimuli in a large contralateral receptive skin field. These units showed latencies within the same range as the units activated from small contralateral receptive fields. The mean value for 9 units with their receptive fields on the forelimb was 12.3 msec (range 10.7—13.9 msec) and the mean value for 11 units activated from the hindlimb was 18.2 msec (range 14.4—21.1 msec).

Units excited from extensive bilateral fields or from ipsilateral fields responded sometimes to electrical stimulation but only with a high voltage shock of 1—2 msec duration. Response to electrical stimulation to peripheral fields of units for which pinching or strong pressure on the skin was the only adequate stimulus was obtained in one unit (DCv 1—10). This unit was activated from the contralateral forelimb by pinching. The latency was 76 msec. In two units activated from hair of contralateral limbs and by strong stimulation on the ipsilateral body and limbs the latency from contralateral forefoot was 17 msec (DCv 3—12) and from contralateral hindfoot 29.7 msec (DC 6—2). The latency of purely ipsilateral fields on forelimb could be measured in 4 units. Three of these units were obtained in DCv preparations and showed latencies of 68 msec (DCv 3—20), 32 msec (DCv 3—18) and 18 msec (DCv 4—18). One unit (DC 8—26) was found in an experiment with only the dorsal columns intact and had a latency of 18.1 msec. In some units activated from large skin fields similar variations of the latency in different parts of the field were obtained as in units with small fields while others showed only small latency changes. Shortening of the latency to increased intensity of the stimulation was also observed but the threshold was too high to allow any systematic study. The mean latency for 5 units activated by vibratory stimuli from the hindlimb was 16.5 msec (range 14.7—18.1 msec).

Discussion

The cortical cells activated *via the dorsal* columns show discharge properties and receptive fields similar to those of the cells studied by GORDON and PAINE (1960) in the nucl. gracilis. Their observation of inhibitory fields adjacent to the excitatory ones in units with small receptive fields

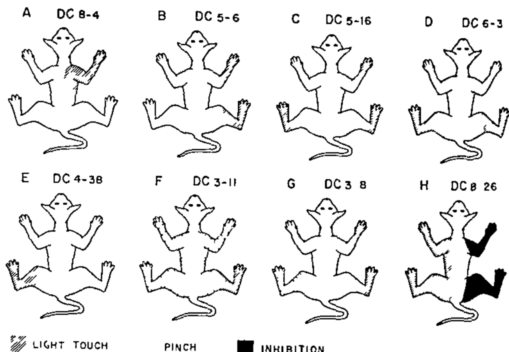


Fig 8 Typical examples of large receptive fields for units obtained in preparations with only the dorsal columns intact The contralateral side of the body corresponds to the right side of the drawings

cells 6 units (3 DC units) were activated only by pinching of the skin or by both skin and deep stimulation Some of the units activated from large fields were also influenced by sound The adaptation to a steady stimulus was fast for most of the units driven from large contralateral fields by light stimuli The units driven by strong stimuli were always slowly adapting Typical also was a slow increase in the firing rate in response to stimulation and a gradual decrease of the discharge after the stimulus was withdrawn

Responses from 16 units (6 DC units) related to deep structures were obtained from large contralateral fields All except one of these units were activated by vibratory stimuli applied with a tuning fork or light tapping The large fields found for these units might in part be due to spread of the stimuli since the vibratory cells were extraordinarily sensitive Several of these units discharged synchronously with the heart beat or discharged to very light tapping on the table or on the frame in which the animal was mounted Response to sound was not found One unit (DC 3—6) was activated by pressure on the muscles below the elbow in the contralateral forelimb

thus confirming the absence of such units in S II (CARRERAS and ANDERSSON 1962). Units in the nucl. gracilis are activated by joint movements (GORDON and SEED 1961; WALL 1961) and such cells are frequently obtained in the thalamic ventral basal complex and in S I (MOUNTCASTLE 1957; MOUNTCASTLE and POWELL 1959 a; ROSE and MOUNTCASTLE 1959; POGGIO and MOUNTCASTLE 1960). The cortical, thalamic and dorsal column nuclei cells activated by gentle rotation of joints are probably functionally connected with joint afferents of large diameter since such fibres mediate responses of low threshold to angular movements and are slowly adapting (BOYD and ROBERTS 1953; SKOGLUND 1956). These fibres are well suited for mediating information about position and movement of the joint. The units in S II responding to joint stimulation are activated by strong stimuli and it is assumed that the effect is mediated by afferents of smaller diameter. The function of these joint afferents is unknown but they have been regarded as a subdivision of the FRA (ECCLES and LUNDBERG 1959).

Although the knowledge about units activated from deep structures is limited, the similarity in fields and discharge properties of the cortical units driven via the dorsal column and the cells studied in the nucl. gracilis by GORDON and PAINE (1960) suggest that the main pattern of organization in the afferent system in the dorsal column pathway mediating information about small fields activated by light stimuli is laid down already at its first relay station.

Cells with large peripheral fields were found by GORDON and PAINE (1960), particularly in the rostral but also in the caudal parts of nucl. gracilis. They observed cells with fields similar to those units with large fields observed in these experiments. They did not report any cells with fields purely contralateral to the investigated nuclei but a few units in the present investigation were found in S II activated only from ipsilateral fields. RAMON Y CAJAL (1896 p. 511) described interconnections between the dorsal column nuclei of both sides. This might explain the bilateral fields in the series of experiments with only the dorsal columns intact. The longer latency and the higher sensitivity to anaesthesia of the ipsilateral response suggests a more complicated pathway. The long distance between the stimulation and recording points and the variability in the latency to identical stimuli make it impossible to draw any conclusions about the number of synapses from the difference in latency between the two sides. There are several possibilities for an ipsilateral cortical effect in these preparations: crossing in the dorsal column or in the relays, an uncrossed ipsilateral pathway from the first relay station or a transcallosal activation from the opposite side of the somatic sensory cortex (cf. ROSE and MOUNTCASTLE [illegible]

is consistent with the topographical relation between excitatory and inhibitory fields of cortical units The results are also in agreement with those of AMASSIAN and DE VITO (1957) KRUGER *et al* (1961) found cells activated from small fields on the distal parts of the contralateral limbs but they could not find any inhibitory effects This discrepancy is explained by KRUGER *et al* (1961) as being due to deep anaesthesia and unsystematic search for this phenomenon At the cortical level it has been observed that *the inhibitory effects are abolished with increased depth of* anaesthesia (MOUNTCASTLE 1957 MOUNTCASTLE and POWELL 1959 b CARRERAS and ANDERSSON 1962) *The finding of longer latency for units* with afferent inhibition deserves *some comment* GORDON and PAINE (1960) found that optimal inhibition in cells in nucl gracilis was obtained when the onset of the inhibitory conditioning stimulus preceeded the excitatory one by 0 to 15 msec following electrical stimulation of branches of peripheral nerves in the contralateral hindfoot and by 0 5—20 5 msec following mechanical stimulation of the receptors The longer latency in units with inhibition might be explained as due to an early onset of inhibition evoked from spread of the electrical stimulus to inhibitory afferents[1] For small excitatory receptive fields such spread might be expected at high intensity of stimulation Lengthening of the latency and decreased propability of firing in some units in response to increased stimulus intensity might be due to recruiting of more inhibitory than excitatory afferents When the stimulus is further increased either inhibition or excitation will dominate giving stronger inhibitory effects or spatial facilitation *with shortening of the latency In units with late onset of the* inhibition a synchronous stimulation of inhibitory and excitatory afferents *can give a gradual shortening of the latency* as the intensity is increased (cf TOWE and KENNEDY 1961)

ARMETT and HUNSPERGER (1961) have emphasized that peripheral interaction at the receptor level can appear as inhibition In the present investigation afferent inhibition has been attributed to a unit only if the spontaneous or evoked response was changed from a field outside the excitatory one Moreover inhibition was not found in units with small receptive fields activated via the lateral tract (see chapter IV)

Units in S II driven from deep structures constitute about 10 % of the total number of units obtained in experiments with only *the dorsal* columns intact None of the units were driven by gentle joint movements

[1] Added in proof McCOMAS (196[illegible]) has recently investigated the nucl *gracilis* in cats with microelectrodes and confirmed that the nucleus has 3 functionally different subdivisions The authors finding of longer latencies in cells in the intermediate region than in cells in the caudal part of the nucleus is probably *due to afferent inhibition* since the intermediate part of the nucleus is *the main relay* for neurones with afferent inhibition (GORDON and PAINE 1960

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Chapter IV

The lateral tract

Introduction

With aid of evoked cortical potentials it has been shown that MORIN's pathway mediates response to light tactile stimulation in S I and S II (MORIN 1955, CATALANO and LAMARCHE 1957, MARK and STEINER 1958, NORRSELL and VOORHOEVE 1962).

Information on the properties of fibres ascending in the dorsal part of the lateral funiculus is available from intra-axonal recordings from second order neurones. These axons could be differentiated from those of the dorsal spino-cerebellar tract because of their origin caudal to CLARKE's column. One group of axons was monosynaptically excited exclusively from ipsilateral (with regard to the spinal recording) cutaneous afferents and adequately activated from small fields by light stimuli without any further increase in response to pressure or pinching of the skin. No activation was obtained from muscle nerves. Another group of axons similarly taking origin caudal to CLARKE's column and ascending in the dorsal part of the lateral funiculus received monosynaptic activation from cutaneous afferents but was in addition activated by volleys in high threshold muscle afferents. On adequate stimulation most of these neurones responded to tactile stimuli and in addition to pressure and pinching in larger areas but some responded only to strong stimuli (LUNDBERG and OSCARSSON 1961). For both types of fibres termination in upper cervical region has been established (with usage of the technique of antidromic stimulation) which suggests a relay in the lateral cervical nucleus (LUNDBERG and NORRSELL 1962 personal communication).

NORRSELL and VOORHOEVE (1962) have shown that axons in the lateral tract conduct effects of tactile stimuli applied to the hindlimb to the cerebral cortex. In the present detailed study of unit activation in S II via the lateral tract it will be shown that effects from skin as well as from deep structures are mediated via this tract and the results will be discussed in relation to the two main types of activation patterns in axons in the lateral tract.

Units activated from large receptive fields present special problems which will be further discussed in chapter VIII

The importance of central influences on ascending spinal pathways (LIVINGSTON 1959) has become increasingly apparent. In particular descending control to the dorsal column nuclei has been demonstrated (WALBERG 1957, DAWSON 1958, SCHERRER and HERNÁNDEZ-PEÓN 1958, GORDON and SEED 1961, JABBUR and TOWE 1961, TOWE and JABBUR 1961, GUZMAN-FLORES *et al.* 1962). Such systems might be of special significance for units activated from large fields since KUYPERS, HOFFMAN and BEASLEY *(1961) found that descending fibers of cortical* origin had a dense termination in those parts of the dorsal column nuclei corresponding to the regions in nucl. gracilis in which units activated from large fields were found by GORDON and PAINE (1960). Transmission at the first synapse in the spinal cord is also subject to a complex supraspinal control (HAGBARTH and KERR 1954, ECCLES and LUNDBERG 1959, HAGBARTH and FEX 1959, HOLMQVIST, LUNDBERG and OSCARSSON 1960, HOLMQVIST and LUNDBERG 1961).

Changes in modality and in the size of the receptive fields found in units in the anterior sigmoid gyrus of the cat (BROOKS, RUDOMIN and SLAYMAN 1961 a, b) and also in some units in this investigation might be related to changes in these control systems.

Summary

The projection to S II via the dorsal columns has been studied with only the dorsal columns intact and with section of the contralateral dorsal *part of the lateral funiculus*.

Most units were activated by light stimuli from small contralateral receptive *fields on the skin. The mean latency for units with small recep*tive fields on the skin was for the forefoot 12.1 msec and for the hindfoot 19.4 msec.

Afferent inhibition was obtained frequently in units with small contralateral receptive fields on the skin: in 35 % of the total population of such units inhibitory effects could be demonstrated from fields surrounding or adjacent to the excitatory one.

Units with *afferent inhibition usually had a longer latency than units* without inhibition. In some units with inhibition lengthening of the latency was observed to increased intensity of the stimulus but more commonly increased intensity caused a shortening of the latency.

9 % of the units related to deep structures were activated from small areas by light or strong stimuli. No units could be influenced by gentle joint movements.

22 % of the units were influenced from large fields. Some of these units were activated only from the skin by light stimuli, other units by both light and strong stimuli. Activation from skin and deep structures as well as from sound was also found.

Some units were activated by vibratory stimuli.

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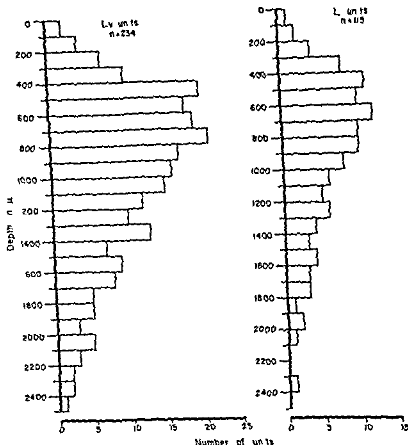


Fig 10 Depth distribution of cortical units studied in preparations with section of only the dorsal columns (left) and in preparations with bilateral hemisections (right). Depth below the pial surface was determined by microscrew readings.

of units was not common when the slow wave potential was still initial positive. Few units were observed more than 2000 μ below the cortical surface. Many penetrations showed only cells of the same modality.

The cells obtained in the two series of experiments are given in Table 4 which shows their distribution with regard to the fields and the modalities "deep" and "skin". Units activated from small contralateral fields constitute the largest groups in both series. The proportion of units activated from contralateral large skin fields was very similar in the two series but the units activated from bilateral and ipsilateral skin fields were found almost exclusively in the preparations with section of only the dorsal columns.

Results

A Material and classification

Cortical effects mediated by pathways outside the dorsal columns were studied in 26 cats The material consists of two series of experiments with different types of spinal lesions In one series (22 experiments) only the dorsal columns were transected at the level of C_{3-4} and 234 units (Lv units) were studied in 66 penetrations The extent of this type of lesion is seen in Fig 9 A In another series the dorsal columns were cut at the same level together with an ipsilateral hemisection This lesion was combined with a contralateral hemisection of the spinal cord at the upper part of C_1, made by ventral approach This type of combined lesion was carried out in 4 experiments in which 115 units (L units) were studied in 21 penetrations In experiment L 2 the lesion of the ipsilateral ventral funiculus was not complete at C_3 The extent of the lesions in these experiments is seen in Fig 9 B and C The location of the penetrations in these two series of experiments is shown in Fig 23 C

Single cells activated by peripheral stimulation were found at all depths of the cortex The distribution in depth is shown in Fig 10 The majority of cells were found 400 μ–1700 μ below the cortical surface with no significant difference in the two series of experiments The cells activated from deep structures and from skin could be found at any depth The superficial layer of the cortex showed very little unit activity and isolation

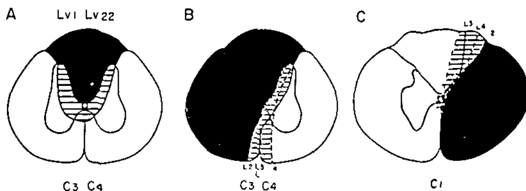


Fig 9 Extent of the spinal cord lesions

A shows the extent of lesions in preparations with section of only the dorsal columns B shows the extent of the hemisection carried out on the ipsilateral (left) side at the level of C_{3-4} in the four experiments with bilateral hemisection and C shows the lesion at upper C_1 on the contralateral (right) side in these experiments Each of these experiments is separately indicated on the figure

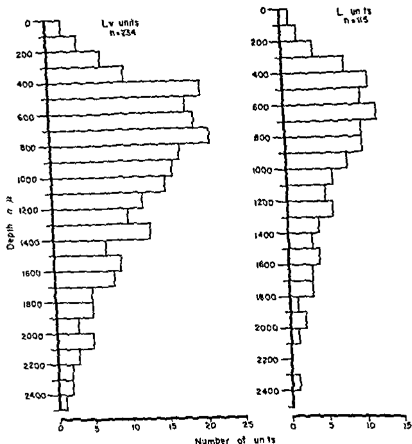


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Table 4

Modality location and extent of the receptive fields for all units (349) studied in L and Lv experiments

Receptive fields		Modality	L Units	Lv units	Totals
Small	contralateral	Skin	84	115	199
		Deep	14	57	71
Large	contralateral	Skin	13	20	33
		Deep	2	9	11
	bilateral	Skin	2	21	23
		Deep	0	3	3
	ipsilateral	Skin	0	8	8
		Deep	0	1	1
		Totals	115	234	349

B *Units activated from small receptive fields*

These units were activated from three types of small receptive fields from skin only, from deep structures only or from light stimuli to the skin but further activated by strong stimuli in a larger field

a *Units activated from small skin areas by light stimuli* The units in this group (188 units) had excitatory fields of similar types to the corresponding group described in chapter III No differences in the properties of these cells were found between the two series of experiments with the lateral tract intact The largest number of units studied were activated from the forelimb, but in other respects hindlimb and forelimb units did not differ Units with small receptive fields on the trunk were not found Most of the units had a low threshold to adequate stimuli such as light touch to pads or hair Blowing of air activated units with their receptive fields in hairy areas and sometimes also units driven from only pads 22 units were activated only by light pressure to the skin The latter units were slowly adapting to a steady stimulus and their receptive fields included hairy areas All other units were fast adapting and fired only at the onset of a steady stimulus Many of the fast adapting units activated from small skin areas had a very low rate of spontaneous discharge and most of the units activated from only pads discharged only in response to stimulation in the receptive fields The slowly adapting units usually fired spontaneously A brief electrical stimulus usually elicited only a few action potentials (Fig 11)

The size of the fields was related to location on the limb On the distal part of the toes some fields covered about 0 5 cm² but larger fields were

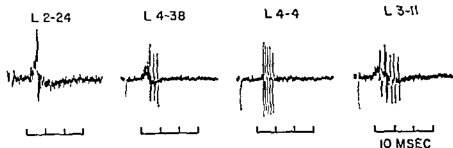


Fig 11 Different types of single cortical unit discharge evoked by high intensity electrical stimulation in the receptive fields of units adequately activated by light stimuli in small fields in preparations with two spinal hemisections Receptive fields of unit L 2—24 — pads and hairy skin contralateral hindfoot, unit L 4—38 — hairy skin, contralateral forefoot, unit L 4—4 — pads and hairy skin, contralateral forefoot and further activated by strong stimuli unit L 3—11 — hairy skin, contralateral forefoot 10 msec between each vertical line of the time marker

A L 2-40 B Lv3-8 C Lv19-11 D L 2-11 E L4-45

F Lv19-23 G L 4-9 H Lv22-8 I Lv22-7 J L4-56

LIGHT TOUCH

Fig 12 Size and location of typical recentiva [illegible]

encountered more proximally (cf MOUNTCASTLE and POWELL 1959 b) The units activated through the lateral tract on the average had somewhat larger fields than the units activated via the dorsal columns and in no case was a unit activated from only a small part of a pad or from a few hairs The most common type of receptive field (79 units) consisted of only hairy skin (Fig 12 C, D, G, H, J) These fields could partly or completely surround one or several pads Fields on the tip of the middle toes of both forefoot and hindfoot were particularly common Fields covering *both the ventral and the dorsal* surface of one or a few toes were more common than fields limited to only the dorsal surface of the paw Fields located on the limb proximal to the foot were less common than in the preparations with the dorsal columns intact Only 18 such units were found and most of these units had fields preaxial on forelimb and *hindlimb*

63 units were activated from both pads and hairy skin (Fig 12 E F, I) and the fields frequently covered several toes Some fields included all the ventral surface of the foot (Fig 12 I)

46 units were related to only pads (Fig 12 A and B) and these units *usually also had receptive fields covering more than one pad and most* commonly including the pads of the middle toes with or without the central pad

b *Units activated from deep structures in small areas* Units activated by receptors below the skin were found more frequently in the preparations with section of only the dorsal columns (Table 4) In both series of experiments 71 units influenced from small areas below the skin were found 57 units (10 L units) were driven from structures in *the contralateral* fore or hindfoot 12 units (4 L units) were driven from the region of a joint proximal to the foot and 2 Lv units were driven from unidentified deep structures in one hindlimb The peripheral fields of the units activated from deep structures in the fore or hindfoot were similar in size to those units driven by *small skin fields Discontinuous* fields were not observed

For some units activated from deep structures the threshold for mechanical stimulation was as low as for units driven from skin and sometimes it was not immediately obvious if a unit was activated from the skin or *from below the skin* This was particularly true for units activated from the distal parts of the limbs The classification of "deep" was made only after careful observations In some units it was nevertheless uncertain if they were activated from deep structures or from *the skin* Such units were classified as "deep" A higher threshold was found for units activated

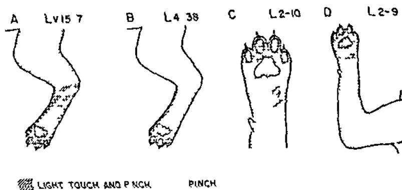


Fig 13 Units activated by light stimuli in small contralateral fields and further activated by strong stimuli in larger fields in preparations with the lateral tract intact C and D represent forelimbs A and B hindlimbs

by receptors related to the region of a joint None of these units responded to gentle movement of a joint The activating stimulus had to be forced movements or pressure to a joint region and it was not possible to localize the effective structures to any particular small area

c *Units activated from small skin areas by light stimuli and further activated by strong stimuli* Eleven units (8 L units) were activated by light stimuli in a small area on the contralateral side and responded also to strong stimuli such as strong pressure or pinching in a larger area The receptive fields in which light stimuli were effective were for all units contralateral and frequently similar to that for units activated only from small cutaneous fields (Fig 13) Outside this area further activation was obtained from both skin and deep structures The fields were continuous and entirely restricted to the contralateral side in all units Most of these units were slowly adapting There was an immediate response to *onset* of the stimulus and no afterdischarge when the stimulus was withdrawn

It was sometimes observed that the rate of adaptation was related to the anaesthesia and when additional anaesthetic was given the adaptation changed from slow to fast

d *Afferent inhibition* Inhibitory effects were looked for in all units In units with spontaneous activity stimuli of different intensities were applied to areas adjacent to the receptive field as well as to other parts of the body but in none of the units activated from small receptive fields was afferent inhibition observed The same result was obtained when inhibition was tested against the background of responses evoked by gentle mechanical stimulation or electrical shocks just above threshold

encountered more proximally (cf MOUNTCASTLE and POWELL 1959 b) The units activated through the lateral tract on the average had somewhat larger fields than the units activated via the dorsal columns and in no case was a unit activated from only a small part of a pad or from a few hairs The most common type of receptive field (79 units) consisted of only hairy skin (Fig 12 C D, G, H, J) These fields could partly or completely surround one or several pads Fields on the tip of the middle toes of both forefoot and hindfoot were particularly common Fields covering both the ventral and the dorsal surface of one or a few toes were more common than fields limited to only the dorsal surface of the paw Fields located on the limb proximal to the foot were less common than in the preparations with the dorsal columns intact Only 18 such units were found and most of these units had fields preaxial on forelimb and hindlimb

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Table 5

Latency changes to changes in the intensity of an electrical stimulus applied in the receptive field for 22 units adequately activated by light stimuli in small fields on contralateral fore (F) or hindfoot (H)

Unit	Latency Threshold Stimulus	Latency Maximum Stimulus	Stimulus × Threshold	Latency Change in msec and %	
L 2—15 F	20.7	10.4	30.0	10.3	99
Lv 14—2 F	13.9	7.4	83.3	6.5	88
Lv 2—13 F	17.2	9.2	42.9	8.0	87
Lv 2—15 F	15.5	8.6	23.0	6.9	80
Lv 20—6 H	21.2	12.0	10.0	9.2	77
Lv 20—16 F	20.7	12.7	74.0	8.0	63
L 2—4a H	25.8	16.8	21.0	9.0	54
L 4—17 F	17.0	11.8	12.5	5.2	44
Lv 14—12 H	15.3	11.5	44.1	3.8	33
L 4—34 H	19.2	14.5	100.0	4.7	32
Lv 18—1 F	17.3	13.5	30.0	3.8	28
Lv 20—18 F	14.0	11.3	3.3	2.7	24
Lv 18—10 H	19.8	16.4	23.0	3.4	21
Lv 14—11 H	14.0	12.0	45.4	2.0	17
Lv 4—4 F	8.6	7.4	6.7	1.2	16
Lv 14—6 H	14.8	12.9	10.0	1.9	15
L 2—38 H	17.4	16.2	63.0	1.2	7
L 3—11B F	15.4	14.4	23.0	1.0	7
L 4—3 H	17.5	17.3	15.0	0.2	1
L 4—29 H	14.4	14.2	10.0	0.2	1
Lv 22—8 H	14.6	14.6	74.0	0.0	0
L 3—11A F	17.0	17.0	23.0	0.0	0

e *Latency measurements* The latency to a maximal electrical stimulus in the receptive field was measured in 87 units adequately activated only by light stimuli to small areas on contralateral fore or hindpaw The distribution of the latencies is shown in the histograms of Fig 14, where the latencies for similar units activated via the dorsal columns also are given (broken lines) The mean latency for 55 units with their receptive fields on the forefoot is 10.5 msec and the mean latency for 32 units with similar fields on the hindfoot is 14.8 msec Inset in the figure are histograms showing the distribution of latencies for the three different types of small fields The number of units in each group is limited but on the average the units activated only by hair have the shortest latency and units activated from only pads the longest latency

In 22 units with small skin fields the latency of the response was measured at different intensities of the electrical stimulation (Table 5) Most of the units showed a decrease in latency to increased intensity of the stimulus Some units showed a rapid decrease in latency when the intensity was raised just above the threshold (Fig 15 L 2—15) and other

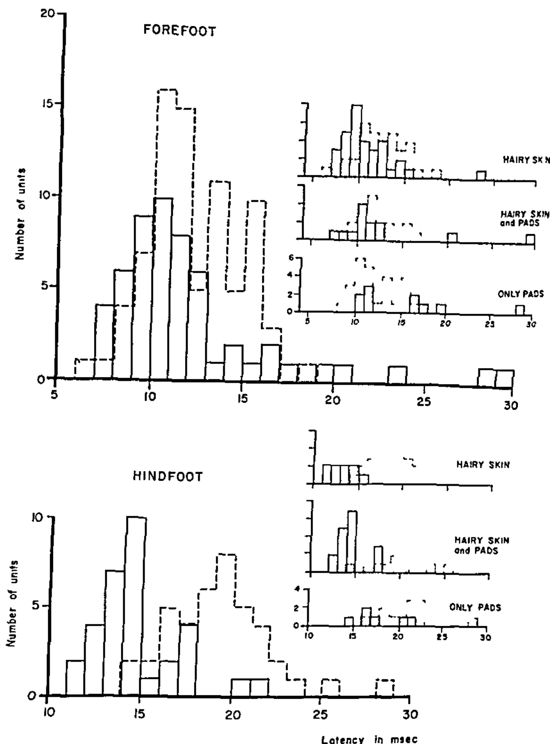


Fig 14 Distribution of the mean latencies for units activated by high intensity electrical stimuli from small receptive fields in the forefoot (55 units) and hindfoot (32 units) in preparations with the lateral tract intact (solid lines) Inset histograms show the distribution of latencies for units with different types of small receptive fields Also shown (dashed lines) are the comparable data obtained for the dorsal column pathway (see Fig 5) Note that the latency axes begin at different values

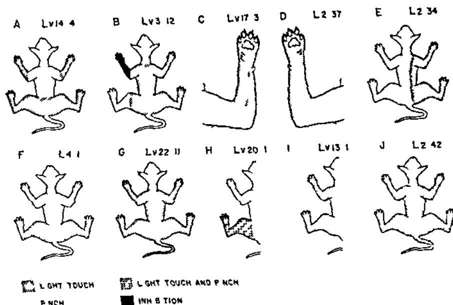


Fig 16 Examples of typical receptive fields of units activated from large areas in preparations with the lateral tract intact In F and J the fields are given for the only two units with bilateral fields found in preparations with two hemisections of the spinal cord The contralateral side of the body corresponds to the right side of the drawings of the animal C represents the ipsilateral forelimb D the contralateral forelimb

Strong pressure or pinching did not give any further activation The receptive fields for 19 units were entirely contralateral and covered frequently both fore and hindlimbs together with some parts of the body (Fig 16 E) or only a stocking like area (Fig 16 D) Six units had bilateral fields (Fig 16 A B and G) In 4 units the receptive fields were ipsilateral (Fig 16 C) Several units were also activated by sound

Only 6 units (1 I unit) were driven from deep structures by light stimuli in large fields All these units were driven by vibratory stimuli Units driven by such stimuli had a threshold as low as those driven via the dorsal columns and the large fields might in part be due to spread of stimulus

b *Units activated from large receptive fields by strong stimuli* 35 units were driven from large skin fields only by strong stimuli such as pinching or strong pressure 29 of these units were found in preparations with section of only the dorsal columns Most units in this group were activated from both skin and deep structures 17 units had bilateral fields covering

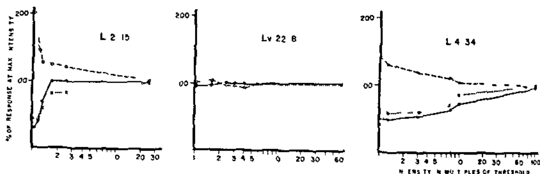


Fig 15 Graphs of the data obtained in intensity series carried out for 3 units in preparations with the lateral tract intact

Open circles and dashed lines — mean latencies solid circles and solid lines — mean number of impulses per response crosses and dotted lines — *probability that the response will occur* Each point is the average of 10—20 single records

units had an almost linear decrease with increased intensity (Fig 15 L 4—34) while others had the same latency independent of the intensity of the stimulus (Fig 15, L 22—8) The latency was also related to different parts of the receptive field and the edges of the fields usually showed longer latency similar to what has been described for the dorsal column preparations

Latency measurements of units activated from deep structures were difficult to obtain Only a few units responded to an electrical shock applied with a pair of needles insulated except on the tips and inserted below the skin Response was obtained only at high voltage and with a stimulus duration of 1—2 msec *The average latency for 6 units activated* from the forelimb was 18 2 msec (range 10 1—27 0 msec) Most units activated by light stimuli in small fields and further activated by strong stimuli responded to electrical shocks within the area giving response to light stimuli The mean latency for 4 units activated from the forelimb was 11 8 msec and 3 units with their fields on the hindlimb had a mean latency of 16 4 msec

C Units activated from large receptive fields

Units with large receptive fields were activated either by light stimuli or by strong stimuli Most of these units were not modality specific

a *Units activated by light stimuli in large fields* 29 units (9 L units all contralateral) were adequately activated from large cutaneous receptive fields by stimuli such as light touch, jets of air or *light pressure*

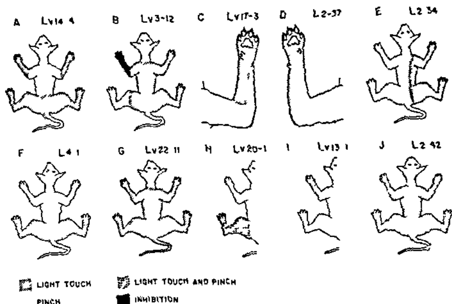


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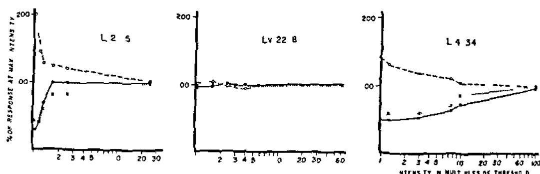


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Table 6

Mean latency in msec for 3 units activated by electrical stimuli applied to the distal part of the 4 limbs

	Forefoot	Hindfoot	Latency difference
Contralateral	17.7	21.8	4.1
Ipsilateral	21.5	2[illegible]	4.2
Latency difference	3.8	3.9	—

be activated from symmetrical points on all feet and the average latency is given in Table 6.

The latency difference between hindlimb and forelimb is in the same range as for units activated from small receptive fields (4.3 msec). The difference between the latency of the contralateral and ipsilateral response is the same for forelimbs as for hindlimbs. This finding indicates that the transmission velocity in the spinal cord is the same for afferents to units with this type of large receptive fields as for units with small contralateral fields. It indicates also that these afferents ascend bilaterally in the spinal cord and project to a more cephalad structure which receives a bilateral input.

Units activated from large fields by strong stimuli did not respond to single electrical stimuli and therefore no latency measurements could be obtained.

Discussion

In the two types of preparations in which the cortical effects via the lateral tract were studied the majority of units were activated from small contralateral receptive fields by light stimuli. MORIN's pathway was originally described as a cutaneous pathway (MORIN 1955). However, the present investigation, particularly the experiments with two complete hemisections of the spinal cord, show that effects from deep structures also are mediated via this tract. Most of the cortical units with small contralateral fields were modality- and place-specific, and thus resemble the modality specific units found by recordings from the second order neurones in the lateral funiculus in cat (LUNDBERG and OSCARSSON 1961), the only difference being that fields including pads are more frequently found in cortical units than in fibres of the lateral tract. The absence of inhibition of the surrounding type in cortical units is also in conformity with the absence of such inhibitory effects in the second order neurones.

An interesting problem is offered by the cortical units which were acti

either symmetrical areas or all the body (Fig 16 F and J) *In some cells* the fields were confined only to the hindbody (Fig 16 I) or to both limbs on one side and one limb on the other side Only two units with bilateral fields were found in the *preparations with two hemisections of the spinal* cord and their receptive fields are shown in Fig 16 F and J 14 units had contralateral receptive fields which in two units covered only the distal *parts of the contralateral forelimb, the other contralateral* units included either one limb with or without parts of the body or the entire side 4 Iv units were activated from only the ipsilateral side and had similar fields *(Fig 16 C H and I)* Several units activated from large fields were also excited by auditory stimuli

One unit (L 2—36) obtained in a preparation with two hemisections of the cord was activated by pressure on a contralateral muscle belly Four other units (Lv 15—5 Lv 20—3, Lv 20—4 and Lv 20—20) were activated by pressure on ipsilateral hindlimb muscles 4 units with bilateral or contralateral receptive fields were activated by strong stimuli but the receptors could not be localized The cells driven by direct pressure on muscles did not respond to stretch of the muscle and there was no indication that the activation was due to stimulation of stretch receptors

The discharge pattern of many of the units driven by strong stimuli *was different from units activated by light stimuli The onset of the stim*ulus did not evoke any immediate effect The discharge rate increased slowly and was proportional to the intensity of the stimulus No adaptation *to a steady stimulus was found and usually the cell fired with a gradual* decrease in frequency for several seconds after the stimulus was withdrawn In two units the receptive fields increased in size after repeated stimulation *In both these units stimulation was effective only in the fore* limbs when the unit first was observed but later, activity could be evoked from the whole animal

c *Afferent inhibition* Inhibitory effects were looked for systematically but were found in 4 units All of them were obtained in preparations with section of only *the dorsal columns In 3 units activated by light or strong* stimuli in large fields the spontaneous and evoked activity could be inhibited by light stimuli to contralateral or ipsilateral areas (Fig 16 B) One unit was inhibited by pressure *on the contralateral forefoot* but no excitatory field was found

d *Latency measurements* Most units activated from large fields by light stimuli did not respond to electrical shocks delivered to the skin in the receptive field or [illegible] constantly 3 units however could

Table 6

Mean latency in msec for 3 units activated by electrical stimuli applied to the distal part of the 4 limbs

	Forefoot	Hindfoot	Latency difference
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Ipsilateral	21 5	2	4 2
Latency difference	3 8	3 9	—

be activated from symmetrical points on all feet and the average latency is given in Table 6

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Discussion

In the two types of preparations in which the cortical effects via the lateral tract were studied the majority of units were activated from small contralateral receptive fields by light stimuli MORIN s pathway was originally described as a cutaneous pathway (MORIN 1955) However the present investigation particularly the experiments with two complete hemisections of the spinal cord show that effects from deep structures also are mediated via this tract Most of the cortical units with small contralateral fields were modality and place specific and thus resemble the modality specific units found by recordings from the second order neurones in the lateral funiculus in cat (LUNDBERG and OSCARSSON 1961) the only difference being that fields including pads are more frequently found in cortical units than in fibres of the lateral tract The absence of inhibition of the surrounding type in cortical units is also in conformity with the absence of such inhibitory effects in the second order neurones

An interesting problem is offered by the cortical units which were acti

vated by light stimuli in small areas and further activated by strong stimuli in larger areas In the spinal tract many neurones showed convergence *of this type* (LUNDBERG and OSCARSSON 1961 LUNDBERG 1962 personal communication) Hence it can be concluded that this subdivision of the spinal tract can be utilized for conduction to the cerebral cortex It should however be noted that in the present investigation units with convergence of different modalities were found mainly in the series of experiments with two hemisections hence in all probability in animals made spinal with respect to descending systems controlling relay stations in the cord The findings of LUNDBERG and OSCARSSON (1961) were made on virtually spinal cats In animals with intact spinal cord convergence of different modalities was not observed in units with small receptive fields in S II (CARRERAS and ANDERSSON 1962) Such convergence might be filtered away by descending inhibitory systems in animals with intact spinal cord possibly in conjunction with selective linkages in higher relay stations Second order neurones characterized by modality convergence in the spinal cord may in the intact animal forward information to cortex only from the most effective source of receptor activation

Units with small receptive fields activated from deep structures were *most common in the preparations with section of only the dorsal columns* Similarly in the experiments in which the projection to S II via the dorsal columns was investigated (cf chapter III) the units related to deep structures were *more common in the preparations with section of only* the dorsal part of the contralateral lateral funiculus than in the experiments with transection of all the cord except the dorsal columns However the existence of a *ventral projection pathway mediating effects from deep structures* in small fields cannot be postulated only on the basis of these findings It must be emphasized that the columnar organization of the cortex should be considered in comparisons of the distribution of units between different preparations A few penetrations can *by* chance give *many units* of one type which thus will be over represented with regard to other types of units

Units with large contralateral receptive fields were equally common in both types of preparations in which the projection to S II via the lateral tract was investigated However units with *bilateral and ipsilateral* receptive fields were almost exclusively found in the experiments with section of only the dorsal columns These differences indicate that the lateral tract receives afferents exclusively or almost exclusively *from the side of* the body in which it ascends in the spinal cord (cf HOLMQVIST and OSCARSSON 1962)

Units with bilateral receptive fields might be *activated by converging*

fibres in MORIN's pathway of both sides at a level cephalad to that of the spinal lesions

The afferent pathway for units with ipsilateral fields is uncertain. An uncrossed ipsilateral tract may exist but in view of the longer latencies of ipsilateral responses the possibility of crossing and recrossing at different levels in the afferent system must be considered (cf Discussion chapter III ROSE and MOUNTCASTLE 1959). It is also possible that some of the units with large receptive fields were activated via pathways ascending in the ventral spinal cord

Units with large receptive fields will be further discussed in chapter VIII

Summary

Projection to S II via the lateral tract was studied in preparations with transection of the dorsal columns at the level of C_{3-4} and in preparations with transection of the dorsal columns and the ipsilateral spinal half at C_{3-4} together with transection of the contralateral spinal cord at the level of upper C_1

Most of the units were activated from small contralateral skin fields similar in size and location to those units activated through the dorsal columns. For none of these units could afferent inhibition be found. The mean latency for units activated from the forelimb was 10.5 msec and from the hindlimb 14.8 msec

20 % of the units were activated from deep structures in small fields by light or strong stimuli. No units responded to gentle movement of joints

Some units were activated by light touch in small fields and further activated by strong stimuli in larger receptive fields

23 % of the units having large receptive fields were activated either by light or strong stimuli. Many units were activated only from the skin but a considerable number both from skin and deep structures. In some of these units interaction between somatic and auditory stimulation occurred. A few units were driven by vibratory stimuli

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20 % of the units were activated from deep structures in small fields by light or strong stimuli. No units responded to gentle movement of joints.

Some units were activated by light touch in small fields and further activated by strong stimuli in larger receptive fields.

23 % of the units having large receptive fields were activated either by light or strong stimuli. Many units were activated only from the skin but a considerable number both from skin and deep structures. In some of these units interaction between somatic and auditory stimulation occurred. A few units were driven by vibratory stimuli.

vated by light stimuli in small areas and further activated by strong stimuli in larger areas. In the spinal tract many neurones showed convergence of this type (LUNDBERG and OSCARSSON 1961, LUNDBERG 1962 personal communication). Hence it can be concluded that this subdivision of the *spinal tract can be utilized for conduction to the cerebral cortex. It should* however be noted that in the present investigation units with convergence of different modalities were found mainly in the series of experiments with two hemisections, hence in all probability in animals made spinal with respect to descending systems controlling relay stations in the cord. The *findings of* LUNDBERG *and* OSCARSSON *(1961)* were made on virtually spinal cats. In animals with intact spinal cord convergence of different *modalities* was not observed in units with small receptive fields in *S II* (CARRERAS and ANDERSSON 1962). Such convergence might be filtered away by descending inhibitory systems in animals with intact spinal cord, possibly in conjunction with selective linkages in higher relay stations. Second order neurones characterized by modality convergence in the spinal cord may in the intact animal forward information to cortex only from the most effective source of receptor activation.

Units with small receptive fields activated from deep structures were most common in the preparations with section of only the dorsal columns. *Similarly, in the experiments in which the projection to S II via the dorsal columns was investigated (cf. chapter III) the units related to deep struc*tures were more common in the preparations with section of only the dorsal part of the contralateral lateral funiculus than in the experiments with transection of all the cord except the dorsal columns. However, the existence of a ventral projection pathway mediating effects from deep structures *in small fields cannot be postulated only on the basis of these find*ings. It must be emphasized that the columnar organization of the cortex should be considered in comparisons of the distribution of units between *different preparations. A few penetrations can by chance give many units* of one type which thus will be over represented with regard to other types of units.

Units with large contralateral receptive fields were equally common in both types of preparations in which the projection to S II via the lateral tract was investigated. However, units with bilateral and ipsilateral receptive fields were almost exclusively found in the experiments with section of only the dorsal columns. *These differences* indicate that the lateral tract receives afferents exclusively or almost exclusively from the side of the body in which it ascends in the spinal cord (cf. HOLMQVIST and OSCARSSON 1962).

Units with bilateral receptive fields might be activated by converging

fibres in MORIN's pathway of both sides at a level cephalad to that of the spinal lesions

The afferent pathway for units with ipsilateral fields is uncertain. An uncrossed ipsilateral tract may exist but in view of the longer latencies of ipsilateral responses the possibility of crossing and recrossing at different levels in the afferent system must be considered (cf. Discussion chapter III, ROSE and MOUNTCASTLE 1959). It is also possible that some of the units with large receptive fields were activated via pathways ascending in the ventral spinal cord.

Units with large receptive fields will be further discussed in chapter VIII.

Summary

Projection to S II via the lateral tract was studied in preparations with transection of the dorsal columns at the level of C_{3-4} and in preparations with transection of the dorsal columns and the ipsilateral spinal half at C_{3-4} together with transection of the contralateral spinal cord at the level of upper C_1.

Most of the units were activated from small contralateral skin fields similar in size and location to those units activated through the dorsal columns. For none of these units could afferent inhibition be found. The mean latency for units activated from the forelimb was 10.5 msec and from the hindlimb 14.8 msec.

20 % of the units were activated from deep structures in small fields by light or strong stimuli. No units responded to gentle movement of joints.

Some units were activated by light touch in small fields and further activated by strong stimuli in larger receptive fields.

23 % of the units having large receptive fields were activated either by light or strong stimuli. Many units were activated only from the skin but a considerable number both from skin and deep structures. In some of these units interaction between somatic and auditory stimulation occurred. A few units were driven by vibratory stimuli.

Chapter V

Further comparison of the cortical effects evoked through the dorsal columns and the *lateral tract*

Introduction

The cortical potentials evoked by a synchronous volley in primary afferents have been used to obtain the outlines of the cortical projection of different parts of the body (ADRIAN 1941 MARSHALL WOOLSEY and BARD 1941) The projection to S II has systematically been studied by WOOLSEY (1947) ADEY CARTER and PORTER (1954) NAKAHAMA (1958) and BERMAN (1961 a) Recently the evoked potential technique has been used to study the cortical projection of different pathways after selective spinal lesions (cf chapters III and IV)

Results

In all experiments large wave potentials were obtained with micro electrode recording in response to a brief peripheral stimulus At the beginning of a penetration this response was used as a gross indication of the part of the body activating the cortical surface below the electrode tip In both types of preparations the wave response was initial positive at the surface but as the electrode penetrated into the cortex the potential gradually changed to an initial negative (cf BREMER 1958 CHANG 1959) There was no obvious difference in the appearance of the slow potentials between the preparations with the dorsal columns or the lateral tract intact (Fig 17) and the behavior of the response was quite similar to that observed in S I with the spinal cord intact (MOUNTCASTLE *et al* 1957)

The average latencies of the slow potentials evoked via the lateral tract and via the dorsal columns are given in Table 7 The difference for the two paths in the latency of the response to stimulation in the contralateral forelimb is only 0 2 msec but the response via the lateral tract has 3 msec shorter latency than through the dorsal columns when the stimulus is applied to the contralateral hindlimb

The latency of single units was frequently related to the latency of the slow wave potentials In penetrations in which the units had long latency

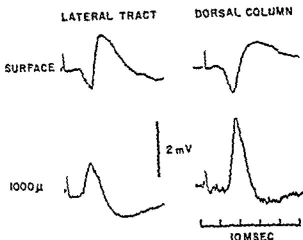


Fig 17 Cortical slow wave potentials obtained to high intensity electrical stimulation of contralateral forefoot at electrode contact with the pial surface and at a depth of 1000 μ in preparations with only the lateral tract intact and with only the dorsal columns intact 10 msec between vertical lines of the time marker

Table 7

Mean latency in msec of intracortical slow wave response obtained by electrical stimulation to hind and forelimbs in preparations with either the dorsal columns or the lateral tract intact

	Forelimb		Hindlimb	
	Contralat	Ipsilat	Contralat	Ipsilat.
Dorsal columns	8 4	13 [illegible]	14 8	19 0
Lateral tract	8 2	12 7	11 8	16 5

the wave potentials had a longer latency than in penetrations with units activated after a short latency The rising negativity of the evoked wave potentials always preceeded the unit discharge by at least 1—2 msec (cf Figs 4 and 11)

The time for complete recovery of the second response to two identical electrical stimuli was studied in some penetrations These studies showed that the dorsal column pathway recovered about 50 msec after a single stimulus and that the recovery was followed by a period of facilitation of the second stimulus lasting for about 50 msec (*Fig* 18 *B*) MORIN's pathway had a slower recovery and had no facilitatory rebound (*Fig* 18 *A*) The recovery of the wave potentials before the spinal lesions were made

Chapter V

Further comparison of the cortical effects evoked through the dorsal columns and the lateral tract

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column pathway when evoked from the contralateral hindlimb but only 0.2 msec shorter when evoked from the forelimb. Measurement of the records of HOLMQVIST and OSCARSSON (1962) reveals that the latency of the mass discharge recorded from the dissected dorsal part of the lateral funiculus at the level of C_3 is 1.3 msec shorter than that in the dissected dorsal columns in response to stimulation of the superficial peroneal nerve but 0.5 msec longer to stimulation of the superficial radial nerve (cf. REXED and STRÖM 1952). The transmission velocity in the dorsal column is 70 m per second (LLOYD and MCINTYRE 1950) and 100 m per second in the lateral tract (LUNDBERG and OSCARSSON 1961). The faster conduction velocity in the lateral tract should account for the shorter latency at C_3 even though the volley in the lateral tract has been delayed at a synapse. Presumably, in the shorter intraspinal course for afferents from the forelimb this synaptic delay has not yet been compensated. It may also be assumed that because of the longer distance from the point of stimulation to the first synapse in the dorsal columns than in the lateral tract a more asynchronous volley is presented to the dorsal column nuclei with a slower rising phase in the excitatory post synaptic potential thereby prolonging the synaptic delay and hence the cortical latency for the dorsal column afferent system. The more pronounced difference between the two pathways in the average latency of the units (see chapter IV) is not contradictory to this interpretation. The units with the shortest latency activated from the forelimb and giving the latency to the slow evoked potential are quite similar for both pathways. The explanation seems to be the occurrence of inhibition in the dorsal column pathway since units with inhibition have a longer overall latency than units without inhibition.

The studies of the recovery period to a single stimulus reveal an absolute refractory period of 10—30 msec. MARSHALL *et al.* (1941) observed that the refractory period was prolonged by anaesthesia and that a summation of the responses to two stimuli with short interval occurred in the awake animal. In single thalamic and cortical units there is an early period of facilitation to a second stimulus (MOUNTCASTLE and POWELL 1959 b, POGGIO and MOUNTCASTLE 1960, MOUNTCASTLE 1961). The length of the absolute refractory period in the present experiments is consistent with a moderate depth of anaesthesia and is similar for both pathways. However, the dorsal column pathway has a shorter relative refractory period which is followed by a period of facilitation to the second response. These differences in the recovery in the two pathways suggest that the dorsal column pathway and MORIN's pathway have different properties with regard to

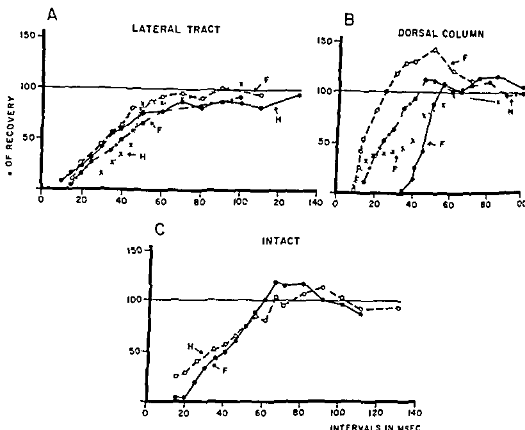


Fig 18 Graphs obtained in studies of the recovery of the wave potential in response to the second of two identical electrical stimuli to the contralateral forepaw (*F*) or hind paw (*H*) in preparations with only the lateral tract (A), only the dorsal columns (B) and the entire spinal cord (C) intact. Each point is the average of approximately 10 single records.

(Fig 18 C) is quite similar to the recovery of the response when only the dorsal columns are intact. The absolute refractory period of 10—30 msec was similar for all the preparations.

Discussion

The difference in latency between MORIN's pathway and the dorsal column pathway to stimulation of the hindlimb found by MARK and STEINER (1958) and by NORRSELL and VOORHOEVE (1962) has been confirmed in this investigation. The difference has been interpreted by the latter authors as due to slower transmission velocity in the first order neurone in the dorsal columns than in the second order neurone in the lateral tract. The present results show that the latency of the cortical response is 3 msec shorter via MORIN's pathway than via the dorsal

column pathway when evoked from the contralateral hindlimb but only 0.2 msec shorter when evoked from the forelimb. Measurement of the records of HOLMQVIST and OSCARSSON (1962) reveals that the latency of the mass discharge recorded from the dissected dorsal part of the lateral funiculus at the level of C_3 is 1.3 msec shorter than that in the dissected dorsal columns in response to stimulation of the superficial peroneal nerve but 0.5 msec longer to stimulation of the superficial radial nerve (cf. REXED and STRÖM 1952). The transmission velocity in the dorsal column is 70 m per second (LLOYD and MCINTYRE 1950) and 100 m per second *in the lateral tract* (LUNDBERG and OSCARSSON 1961). The faster conduction velocity in the lateral tract should account for the shorter latency at C_3 even though the volley in the lateral tract has been delayed at a synapse. Presumably in the shorter intraspinal course for afferents from the forelimb this synaptic delay has not yet been compensated. It may also be assumed that because of the longer distance from the point of stimulation to the first synapse in the dorsal columns than in the lateral tract a more asynchronous volley is presented to the dorsal column nuclei with a slower rising phase in the excitatory post synaptic potential thereby prolonging the synaptic delay and hence the cortical latency for the dorsal column afferent system. The more pronounced difference between the two pathways in the average latency of the units (see chapter IV) is not contradictory to this interpretation. The units with the shortest latency activated from the forelimb and giving the latency to the slow evoked potential are quite similar for both pathways. The *explanation seems* to be the occurrence of inhibition in the dorsal column pathway since units with inhibition have a longer overall latency than units without inhibition.

The studies of the recovery period to a single stimulus reveal an absolute refractory period of 10—30 msec. MARSHALL *et al.* (1941) observed that the refractory period was prolonged by anaesthesia and that a summation of the responses to two stimuli with short interval occurred *in the awake* animal. In single thalamic and cortical units there is an early period of facilitation to a second stimulus (MOUNTCASTLE and POWELL 1959 b, POGGIO and MOUNTCASTLE 1960, MOUNTCASTLE 1961). The length of the absolute refractory period in the present experiments is consistent with a moderate depth of anaesthesia and is similar for both pathways. However the dorsal column pathway has a shorter relative *refractory* period which is followed by a *period of facilitation* to the second response. These differences in the recovery in the two pathways *suggest that the* dorsal column pathway and MORIN's pathway have different properties with regard to

synaptic transmission possibly related to the occurrence of afferent inhibition in the dorsal column pathway.[1]

Summary

Similar evoked potential waves were obtained to a brief peripheral stimulus through the dorsal columns and the lateral tract.

The latency of the response to stimulation of the hindlimb was 3 msec shorter when evoked via the lateral tract than via the dorsal columns. Stimulation of the forelimb gave insignificant latency differences.

A larger difference between the average latency for units of the two pathways is interpreted as due to inhibition in the dorsal column pathway and not in MORIN's pathway.

The absolute refractory period is similar for both pathways but the dorsal column pathway has a shorter relative refractory period followed by a facilitatory rebound.

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[1] Added in proof: ANDERSEN, ECCLES and SCHMIDT (19[illegible]) have found presynaptic inhibition in nucl. cuneatus in response both to stimulation of afferent nerves and to stimulation of the sensorimotor cortex. The possibility exists that the cortical facilitatory [illegible] in the present experiments (see Fig. 18 B and C) is a result of a rel[illegible]

TER ENGBERG and LUNDBERG 19[illegible], personal communication.

Chapter VI

Direct spino-cerebellar pathways

Introduction

GOLDMAN and SNIDER (1955) found that stimulation of cerebellar afferents elicited a response in the cerebellar efferents in the brachium conjunctivum through monosynaptic and polysynaptic pathways. Stimulation of cerebellar structures evokes within a few msec responses in the cerebral sensory somatic cortex (HENNEMAN *et al.* 1952, COMBS and SAXON 1959). Hence it is possible that a response of short latency in the cerebral somatic cortex could be mediated via the dorsal and the ventral spino-cerebellar tracts. Of particular interest in this context is the finding that some subgroups of the dorsal spino-cerebellar tract are activated from exteroceptors (LUNDBERG and OSCARSSON 1960).

Results

In order to investigate effects in the cerebral cortex that could be mediated via direct spino-cerebellar tracts the dorsal part of the lateral funiculus was sectioned on the contralateral side at the level of L_2 together with the dorsal roots of L_1 and L_3. The dorsal columns were transected at the level of Th. The extent of the lesions is shown in Fig. 19. These lesions interrupt the specific projection pathways to the cerebral cortex but leave the dorsal spino-cerebellar tract (DSCT) and practically all of its inflow from the hindlimbs intact. These lesions do not interfere with the ventral spino-cerebellar tract (VSCT) (OSCARSSON 1957).

Evoked responses in S II were looked for in three experiments after the spinal lesions mentioned above. In two cats S II was systematically investigated with surface electrode and the responses to adequate and electrical stimulation of the hindlimbs were studied. No evoked responses of short latency were found to stimulation of the hindlimbs but were regularly obtained to stimulation in the forelimbs and to auditory stimuli in the corresponding cortical regions. In one cat S II was studied with microelectrode recording. The units found showed an activity similar to what will be described in the section on the cortical effects of ventral

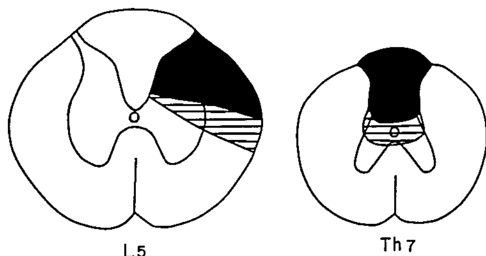


Fig. 19. Extent of spinal lesions at the level of L5 and Th7 in preparations in which cortical effects mediated via direct spino-cerebellar pathways were looked for.

spinal pathways (chapter VII). No units were driven by light adequate stimuli or by single electrical shocks to the hindlimbs but driving with strong stimulation in this region was frequently seen.

Discussion

The Group I muscle afferents project via DSCT and VSCT to cerebellum (OSCARSSON 1956, LUNDBERG and OSCARSSON 1960) and most investigators agree that there is no projection from these afferents to the somatic cerebral cortex (for references see ROSE and MOUNTCASTLE 1959, cf. however AMASSIAN and BERLIN 1958).

The present results show that no effects of short latency in response to *light mechanical or electrical stimulation to the skin of the hindlimbs* are mediated via direct spino-cerebellar pathways and cerebellum to S II. COMBS and SAXON (1959) could not confirm the high sensitivity of the cerebellar cortex to barbiturates described by HENNEMAN *et al.* (1952) but this finding does not exclude that there are functionally important short latency connections from the periphery to the cerebral cortex via cerebellum in the awake state. Although it cannot be excluded that the units driven by strong stimuli to the hindlimbs might have been driven via direct spino-cerebellar pathways it seems much more likely that these units were activated via the ventral spinal pathways, since they had fields and discharge properties quite similar to those obtained in units in preparations with only the ventral spinal cord intact (chapter VII).

Summary

Projection to the cerebral cortex from the hindlimbs via cerebellum has been studied after transection of the specific projection pathways but with the dorsal and ventral spino cerebellar pathways intact

No short latency effects from the hindlimbs could be demonstrated in S II

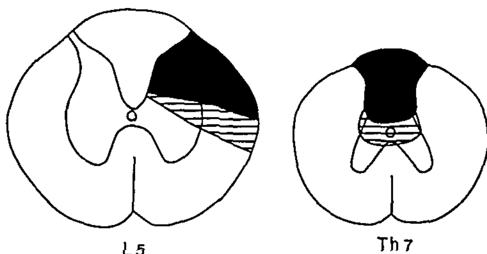


Fig 19 Extent of spinal lesions at the level of L5 and Th7 in preparations in which cortical effects mediated via direct spino cerebellar pathways were looked for

spinal pathways (chapter VII) No units were driven by light adequate stimuli or by single electrical shocks to the hindlimbs but driving with strong stimulation in this region was frequently seen

Discussion

The Group I muscle afferents project via DSCT and VSCT to cerebellum (OSCARSSON 1956 LUNDBERG and OSCARSSON 1960) and most investigators agree that there is no projection from these afferents to the somatic cerebral cortex (for references see ROSE and MOUNTCASTLE 1959 cf, however, AMASSIAN and BERLIN 1958)

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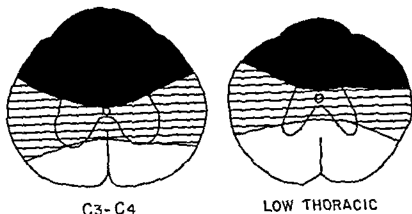


Fig 20 Extent of the spinal lesions at the level of C_{3-4} and in the thoracic region in experiments in which the cortical effects mediated via ventral pathways were investigated

of C_{3-4} or in low thoracic spinal cord The extent of the lesions is seen in Fig 20 In 27 penetrations in S I and S II 183 units were influenced by peripheral stimulation below the level of the spinal lesion 70 units were studied in the cortical areas corresponding to the projection from fields below the spinal lesion These cortical areas have been defined as the "denervated" cortex in contrast to the "innervated" cortex comprising the cortical projection areas with all their afferent pathways intact (ANDERSSON 1962 a) 113 units were studied in the "innervated" cortex

The "denervated" cortex is characterized by the appearance of high amplitude slow waves with a spontaneous frequency of 8—12 per second in an area strictly limited to the projection area with its peripheral fields below the lesion (ANDERSSON 1962 a) The discharge type was the same for most cells and very different from cells in the "innervated" cortex The spontaneous activity of the cells consisted of bursts (Fig 21 B) frequently at the most negative peak of the slow waves but in some units apparently unrelated to the waves The overall frequency also changed periodically Higher rate occurred in periods with larger slow waves

The frequency of the unit discharges could in no case be influenced by light peripheral stimuli such as *jets of air bending of hairs* or by single electrical shocks In addition short *latency slow* evoked potentials could not be observed in response to such stimulation The effective stimulus had always to be strong i e pinching of skin or pressure on skin or structures below the skin Pressure on exposed muscles was very effective for many units other units responded to stimulation in joint regions

Chapter VII

Pathways in the ventral spinal cord

Introduction

In chapters III and IV it was shown that short latency responses of units in somatic sensory cortex in cat are mediated via the dorsal columns and the lateral tract. However, in preparations with intact ventral spinal cord units with large peripheral fields excited by strong stimuli are more numerous than in preparations with only one of the short latency pathways intact. This difference suggests that cortical cells are influenced via pathways outside these short latency tracts. Cells activated by strong stimuli in large fields have been found both in the posterior group of thalamic nuclei and in other thalamic structures in cat and monkey after section of the dorsal funiculi (GAZE and GORDON 1955, WHITLOCK and PERL 1959, KRUGER and ALBE FESSARD 1960, POGGIO and MOUNTCASTLE 1960).

Axonal recording from fibres in the ventral spinal quadrants has revealed fibres activated by cutaneous, muscular and joint afferents in large receptive fields (OSCARSSON 1958). One type of these fibres has bilateral receptive fields and has been traced to the level of the inferior colliculi. Another type of fibres is activated by large contralateral fields (LUNDBERG and OSCARSSON 1962). The transmission to these tracts is subject to an inhibitory supraspinal control (HOLMQVIST *et al.* 1960).

In preliminary reports (ANDERSSON 1962 a, b) localized release of high voltage slow waves in both S I and S II after section of the specific pathways projecting to these areas was described. It was also found that waves and units could be driven via the remaining ventral pathways. The hypothesis was put forward that activity in the specific projection pathways has an inhibitory effect on the mechanism responsible for high voltage slow cortical waves.

Results

Effects on the somatic sensory cortex through pathways ascending in the ventral spinal cord were studied in 18 cats. The dorsal columns and the dorsal part of the lateral funiculi were transected either at the level

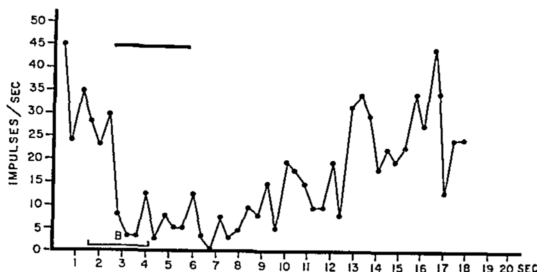
in animals with intact spinal pathways. Thus in an experiment with a cervical spinal lesion units obtained in the cortical hindlimb areas were best driven from the hindlimbs. The units were very seldom influenced by stimulation above the level of the spinal lesion but 4 units found in preparations with a low thoracic lesion were *activated from the entire animal*.

The units studied in the "denervated" cortex were slowly *adapting*. In most of them a delay of hundreds of msec from onset of the stimulus *to the first change in the frequency* was found. Usually the units were *excited* by the stimulus. The frequency increased during stimulation and showed a long afterdischarge. Grouped action potentials (bursts) were typical. 11 units were *inhibited* and these units also showed a long post stimulatory effect (Fig. 21).

Most of the units studied in the "innervated" cortex were obtained in the projection areas of the forelimb in S I and S II after a low thoracic lesion. In the "innervated" cortex the EEG recorded with microelectrode did not differ from what is found in preparations with intact spinal cord and showed wave activity with occasional spindle bursts and immediate and shortlasting desynchronization of the wave activity in response to *light stimulation of the contralateral forelimb*. *Single units were activated* from the contralateral forelimb as is typical for preparations with intact spinal pathways (Fig. 29 B). Similar stimuli applied to the hindlimbs did not evoke any change in the EEG or in the unit activity. However strong stimuli such as pinching of the skin strong pressure on structures below the skin or forced movements of a joint influenced unit activity (Fig. 29 A). The latency of the response was long and the post stimulatory effect *considerable*. Most units were excited but in 5 units the spontaneous discharge was inhibited by strong stimulation in fields below the lesion. Units with this activation pattern were also found outside the *somatic sensory* cortex. In contrast to the bursty activity of the units in the "denervated" cortex the units in the "innervated" cortex showed a fairly regular discharge. The effect obtained from stimulation below the level of the lesion was very sensitive to anaesthesia if more Nembutal was given the driving disappeared.

In the "innervated" cortex the cells influenced by strong stimulation in large fields below *the cord lesion comprised* some 50 % of the total number of cells activated by light stimulation of the forelimb. Both types of units (with and without convergence) were randomly *intermingled* and their discharge properties in response to light stimulation did not differ. This convergence was observed both in cells activated by light stimuli

A



B



Fig 21 Microelectrode recording showing inhibition of a unit found in the hindlimb region in S I after transection of the dorsal half of the spinal cord at the level of C_4 Effect of pressure on the ipsilateral hindlimb

A Graph of the unit discharge frequency plotted against time Horizontal bar indicates duration of the stimulus The horizontal line labelled B represents that part of the record pictured in B below

B Oscillographic record of the unit discharge including the onset of the stimulus (white horizontal line) Note that the spontaneous discharge occurs in bursts and that several units with discharges of low amplitude are activated by the stimulus

either to pressure on the joint capsule or to hyperextension or strong rotation of the joint Gentle movements were ineffective or gave only slight activation *The receptive field was usually very large In preparations* with the spinal lesion in the cervical cord many units were influenced from the whole body Stimulation on the contralateral side was for most units more effective than ipsilateral stimulation There was a tendency to obtain the best driving from the part of the body which normally would have activated the cortex at the position of the electrode

and STEINER 1958, NORRSELL and VOORHOEVE 1962). However cortical activation can still be elicited through the remaining ventral pathways: the long latency, the long time elapsing before maximum response, the long post-stimulatory effect and the sensitivity to anaesthesia of both waves and single units indicate that complicated polysynaptic pathways are involved. The widespread cortical effects obtained via the ventral spinal cord seem to be quite different from those evoked through the specific projection pathways. In preparations with intact spinal cord units activated from large fields by strong stimuli are few in S I (MOUNTCASTLE and POWELL 1959 b) but more common in S II (CARRERAS and ANDERSSON 1962). After large lesions in the dorsal spinal cord including section of the specific projection pathways units driven by strong stimuli in large fields were very common in both S I and S II and such units were also found outside the somatic sensory cortex.

ANDERSSON (1962 b) has shown that the ventral bilateral flexion reflex tract (bVFRT) (LUNDBERG and OSCARSSON 1962) activates cortical units and induces an increased frequency and amplitude of the slow waves. The bVFRT is polysynaptically activated from the FRA and has been traced up to the level of the inferior colliculi by LUNDBERG and OSCARSSON (1962). The further connections of this pathway are unknown.

It is not possible to state if in the intact animal this pathway contributes to the cortical activation of units with large receptive fields or to the projection to the posterior group of thalamic nuclei described in cat by WHITLOCK and PERL (1959). However, the fields and discharge properties of units in the posterior group of nuclei (WHITLOCK and PERL 1959, POGGIO and MOUNTCASTLE 1960) are similar to those found from fibre recording in the ventral spinal cord (LUNDBERG and OSCARSSON 1962).

HOLMQVIST *et al.* (1960) showed that descending pathways in the dorsal part of the lateral funiculi exert a tonic inhibition on the transmission from FRA to ascending tracts. In the present experiments these descending inhibitory pathways were cut. This would give an increased ascending transmission in the ventral pathways activated by such afferents. The hypothesis that the specific pathways exert an inhibitory influence on the mechanism responsible for the slow waves in the intact animal (ANDERSSON 1962 b) also includes the possibility that this mechanism inhibits the unit activation seen in these experiments. The occurrence of units with bursty activity frequently related to the slow waves favors this opinion. Thus it seems reasonable to assume that in the intact spinal cord there are at least two mechanisms controlling the effects ascending from the ventral spinal tracts: 1) descending tracts with tonic inhibitory effect at the first relay, 2) inhibitory effects from ascending

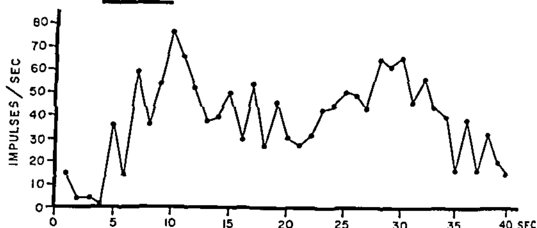




Fig 22 Microelectrode recording of a unit in the forelimb region in S I in a preparation with transection of the dorsal half of the spinal cord at the level of Th_{10}

A Graph of frequency of action potentials plotted against time in response to pinching of the contralateral hindlimb Note that the frequency has not yet returned to resting level 30 sec after cessation of the stimulus

B Oscillographic record of the discharge of the same unit showing the response to a jet of air to a small receptive field in the contralateral forefoot

Horizontal lines in A and B indicate duration of stimulation

to small skin fields and by gentle joint movement (joint units appeared only in S I)

One of the ventral pathways mediating the cortical effect is identified as the bVFRT of LUNDBERG and OSCARSSON (1962) (ANDERSSON 1962 b)

Discussion

The absence of evoked potentials of short latency to brief adequate stimuli and to a single electrical shock after sectioning of the dorsal columns and the lateral tract is in agreement with similar findings in earlier investigations (MORIN 1955 CATALANO and LAMARCHE 1957 MARK

and STEINER 1958 NORRSELL and VOORHOEVE 1962) However cortical activation can still be elicited through the remaining ventral pathways the long latency the long time elapsing before maximum response the long post stimulatory effect and the sensitivity to anaesthesia of both waves and single units indicate that complicated polysynaptic pathways are involved The widespread cortical effects obtained via the ventral spinal cord seem to be quite different from those evoked through the specific projection pathways In preparations with intact spinal cord units activated from large fields by strong stimuli are few in S I (MOUNTCASTLE and POWELL 1959 b) but more common in S II (CARRERAS and ANDERSSON 1962) After large lesions in the dorsal spinal cord including section of the specific projection pathways units driven by strong stimuli in large fields were very common in both S I and S II and such units were also found *outside* the somatic sensory cortex

ANDERSSON (1962 b) has shown that the ventral bilateral flexion reflex tract (bVFRT) (LUNDBERG and OSCARSSON 1962) activates cortical units and induces an increased frequency and amplitude of the slow waves The bVFRT is polysynaptically activated from the FRA and has been traced up to the level of the inferior colliculi by LUNDBERG and OSCARSSON (1962) The further connections of this pathway are unknown

It is not possible to state if in the intact animal this pathway contributes to the cortical activation of units with large receptive fields or to the projection to the posterior group of thalamic nuclei described in cat by WHITLOCK and PERL (1959) However the fields and discharge properties of units in the posterior group of nuclei (WHITLOCK and PERL 1959 POGGIO and MOUNTCASTLE 1960) are similar to those found from fibre recording in the ventral spinal cord (LUNDBERG and OSCARSSON 1962)

HOLMQVIST *et al* (1960) showed that descending pathways in the dorsal part of the lateral funiculi exert a tonic inhibition on the transmission from FRA to ascending tracts In the present experiments these descending inhibitory pathways were cut This would give an increased ascending transmission in the ventral pathways activated by such afferents The hypothesis that the specific pathways exert an inhibitory influence on the mechanism responsible for the slow waves in the intact animal (ANDERSSON 1962 b) also includes the possibility that this mechanism inhibits the *unit* activation seen in these experiments The occurrence of units with bursty activity frequently related to the slow waves favors this opinion Thus it seems reasonable to assume that in the intact spinal cord there are at least two mechanisms controlling the effects ascending from the ventral spinal tracts 1) descending tracts with tonic inhibitory effect at the first relay 2) inhibitory effects from *ascending*

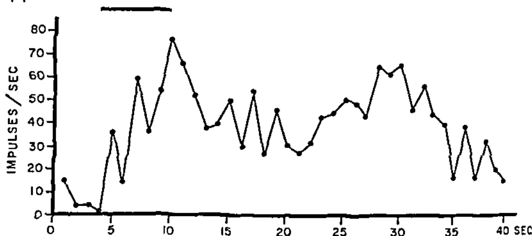




Fig 22 Microelectrode recording of a unit in the forelimb region in S I in a preparation with transection of the dorsal half of the spinal cord at the level of Th_{10}

A Graph of frequency of action potentials plotted against time in response to pinching of the contralateral hindlimb Note that the frequency has not yet returned to resting level 30 sec after cessation of the stimulus

B Oscillographic record of the discharge of the same unit showing the response to a jet of air to a *small receptive field in the contralateral forefoot*

Horizontal lines in A and B indicate duration of stimulation

to small skin fields and by gentle joint movement (joint units appeared only in S I)

One of the ventral pathways mediating the cortical effect is identified as the bVFRT of LUNDBERG and OSCARSSON (*1962*) (ANDERSSON *1962 b*)

Discussion

The absence of evoked potentials of short latency to brief adequate stimuli and to a single electrical shock after sectioning of the dorsal columns and the lateral tract is in agreement with similar findings in earlier investigations (MORIN 1953 CATALANO and LAMARCHE 1957 MARK

Chapter VIII

Further observations on cortical effects elicited from large peripheral areas

Introduction

In a single unit analysis of the projection to S II in cats with intact spinal cord CARRERAS and ANDERSSON (1962) found that response to bilateral stimulation and units activated by strong stimuli were most common in the posterior part of anterior ectosylvian gyrus on the border of the second auditory area S II is a composite cortical area receiving not only somatic projection but also input from the auditory and vestibular systems (TUNTURI 1945 WALZL and MOUNTCASTLE 1949 MICKLE and ADES 1952 ANDERSSON and GERNANDT 1954 BERMAN 1961 a b)

Results

The location of penetrations giving responses from forebody and hind body was confined within the limits of S II as determined by cortical maps of the evoked surface positive potential *in cat* (WOOLSEY 1947 BERMAN 1961 a) and no obvious difference was found in cortical localization between the responses mediated by the dorsal column pathway and the pathway of MORIN Short latency evoked potentials from large areas and units with large receptive fields activated by several modalities were particularly common in the posterior part of anterior ectosylvian gyrus on the border of the second auditory area In this region responses from widely separated parts of the body were obtained via both the dorsal columns and the lateral tract as is indicated *in Fig* 23 B and C In no penetration were responses evoked exclusively from the ipsilateral side and the amplitude of the wave potentials was usually *considerably* less when evoked from the ipsilateral than from the *contralateral* side (cf NAKAHAMA 1961) Units with bilateral fields were most frequently found intermingled with units driven *from only the* contralateral side In many penetrations evoked potentials as well as unit *activation* were obtained in response both to somatic and to auditory *stimulation* In the region of S II where responses from large fields were *obtained* other penetrations showed only units and evoked slow *potentials* activated from small fields

specific projection pathways at higher levels. For these reasons these effects obtained via the ventral pathways cannot be compared with the effects found when both the ascending inhibitory control exerted by the specific projection pathways and the descending control system of HOLMQVIST *et al* (1960) are active.

The possibility exists that other ventral pathways than the bVFRT contribute to the activation of units with large receptive fields in S II. LUNDBERG and OSCARSSON (1962) have raised the question if the contralateral ventral flexion reflex tract (cVFRT) may be a spino-thalamic tract. If so, this pathway is an obvious candidate for such activation. On the other hand the anatomical finding that the spino-thalamic tract terminates in the ventral basal complex (ANDERSON and BERRY 1959) is not in agreement with the possibility discussed in the next chapter, namely that the posterior group of thalamic nuclei relay modality unspecific effects via ventral pathways to cortical units with large receptive fields.

Summary

The cortical effects mediated via ventral spinal pathways have been investigated after section of the dorsal columns and the dorsal part of the lateral funiculi. In cortical areas representing peripheral fields below the level of the cord lesion unit activity appeared in bursts frequently related to cortical waves. The activity of many units can be increased by strong stimuli to large bilateral fields below the spinal lesion. The effect of the stimulus appeared after a long latency, was longlasting and was very sensitive to anaesthesia.

In cortical areas with the specific projection system intact many units activated by light stimuli in small peripheral receptive fields above the spinal lesion were also effected by strong stimuli below the lesion in a similar way as units in the cortical area corresponding to the projection from below the lesion. These findings are discussed in relation to mechanisms controlling the ascending activity in ventral spinal pathways.

Chapter VIII

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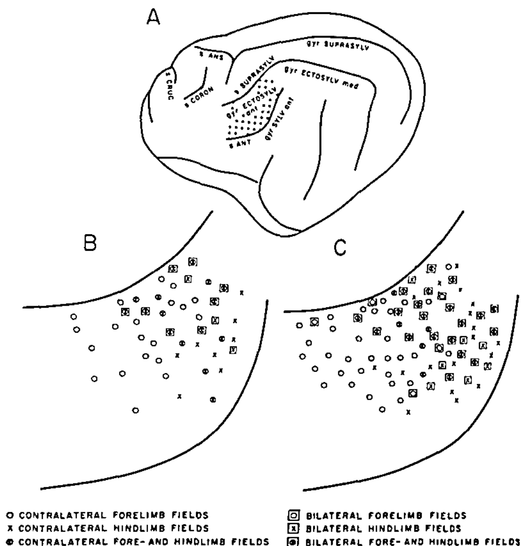


Fig 23 Distribution of penetrations in S II

A Schematic drawing of the cerebral cortex of cat with the area investigated in these experiments stippled

B and C Schematic representation of anterior ectosylvian gyrus

B Distribution of 47 penetrations made in both series of experiments with dorsal columns intact

C Distribution of 87 penetrations made in both series of experiments with the lateral tract intact

In B and C each point was classified according to the maximum peripheral field as judged by both single units and evoked potential waves at all levels in the penetration

on the contralateral side These units were modality- and place specific In the more rostral part of the anterior ectosylvian gyrus (forelimb area) responses in practically all penetrations were only from the contralateral side

The units with large receptive fields or with convergence of different modalities showed a high susceptibility to anaesthesia and for some of these units the receptive fields decreased in size when more anaesthesia was given.

Discussion

The distribution of the penetrations in S II demonstrates that responses evoked from large areas are mainly obtained in the posterior part of the anterior ectosylvian gyrus. Such responses were obtained *via* both the dorsal columns and the lateral tract although only two units showed bilateral fields in the preparations with two hemisections of the cervical cord. In the same region of S II other penetrations showed responses only from contralateral small fields. A more anterior part of the gyrus is almost exclusively activated from the contralateral forelimb. These findings are in agreement with those of CARRERAS and ANDERSSON (1962).

Gross electrode and single unit recordings in thalamic nuclei have shown that almost all the cells in the ventral basal complex have properties similar to those cells in S I and S II activated with short latency from small contralateral fields. These thalamic cells are shown also to be place and modality specific (ROSE and MOUNTCASTLE 1959; POGGIO and MOUNTCASTLE 1960). Although a few units activated from large fields and with longer latency have been found in these nuclei (GAZE and GORDON 1954; MALLART, MARTINOYA and ALBE-FESSARD 1961) cells activated from large fields by strong stimuli or by several modalities are common in the posterior group of thalamic nuclei (WHITLOCK and PERL 1959; KRUGER and ALBE-FESSARD 1960; POGGIO and MOUNTCASTLE 1960). The posterior group of nuclei as well as the centrum median parafascicular complex (ANDERSON and BERRY 1959; KRUGER and ALBE-FESSARD 1960; ALBE-FESSARD and KRUGER 1962) have been considered as a relay for fibres ascending in the ventral spinal cord (cf. chapter VII). The ventral basal complex has been shown to project to S I and S II (KNIGHTON 1950; ROSE and WOOLSEY 1958; MACCHI *et al.* 1959). Degenerations found in the posterior group of nuclei particularly after cortical lesions including the posterior part of the anterior ectosylvian gyrus indicate that these nuclei have a projection to this cortical area (DIAMOND, CHOW and NEFF 1958; ROSE and WOOLSEY 1958). Histological studies of the brain stem have shown that in addition to collaterals to the reticular formation some fibres leave the medial lemniscus before its termination in the thalamic ventral basal complex. PROBST (1902) described fibres from the medial lemniscus entering the medial geniculate body and MATRICALI (1961) has described

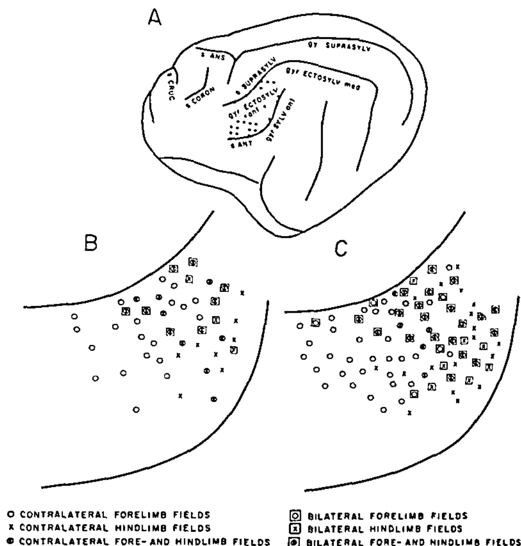


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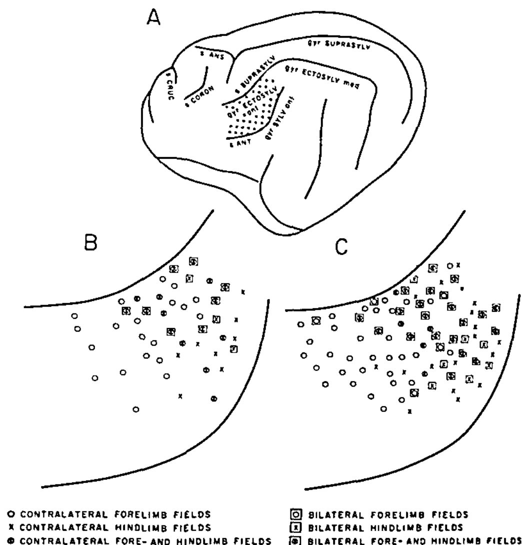


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Chapter IX

Comments and summary

This investigation is concerned with the existence and properties of projection to single *cells in the second somatic sensory* area in cat via the following ascending spinal pathways the *dorsal columns* (chapter III) the *lateral tract* (chapter IV) direct *spino cerebellar pathways* (chapter VI) and *ventral pathways* (chapter VII)

The results confirm the earlier observation that cortical responses of short latency in cat are mediated via two spinal pathways 1) the dorsal columns 2) the lateral tract which is the spinal part of MORIN s pathway Through both these projection pathways the majority of cortical units are excited from small skin fields on the distal part of the contralateral limbs The properties of such cortical units activated via the dorsal *column* pathway and via MORIN s pathway are compared with the responses of those found in the second order neurone The results *suggest that* the modality specificity and the size of the *receptive fields for different* types of cortical units are largely determined already at the first *relay* stations of the two pathways The modality *convergence observed* in one type of second order neurones in the lateral tract has been recognized under special circumstances also in cortical units The *possibility* that a filtration mechanism en route to cortex can transform the message to a modality specific one is discussed in chapter IV

The most pronounced difference between the two pathways with regard to the small skin fields is the occurrence of *inhibition* in the dorsal column pathway and not in the pathway of MORIN In the total [illegible] of [illegible] st [illegible] o [illegible] %. *The inhibitory* field *surrounds or is adjacent to the excitatory* one In none of the units with small fields activated via the lateral tract *could such afferent* inhibition be found This difference between the dorsal *column* pathway and MORIN s pathway suggests that they have *different* functional significance From clinical evidences in man (FOERSTER *1936* pp 3a9—367 and others) it has been suggested that the dorsal column pathway has an

such termination for fibres from the nucl. cervicalis lateralis in the rabbit. RANSON and INGRAM (1932) found that fibres originating in the dorsal column nuclei reach the inferior colliculi and a similar termination was observed by BUSCH (1961) for fibres taking origin from lateral cervical nucleus (cf. however BOWSHER 1958). STARZL, TAYLOR and MAGOUN (1951) describe collateral activation in nucl. lateralis posterior and other parts of the posterior thalamus to stimulation of the sciatic nerve. It thus seems reasonable to assume that some fibres from the medial lemniscus relay in the posterior group of thalamic nuclei. POGGIO and MOUNTCASTLE (1960) found somatic and auditory interaction in these nuclei and such interaction found in posterior S II at unit level is both of cortical and subcortical origin (CARRERAS and ANDERSSON 1962). The fields and discharge properties of the cells in these nuclei are quite similar to those of the units with large fields in the posterior part of the anterior ectosylvian gyrus. In this connection it is interesting that cortical units with trigeminal innervation fields are more frequently activated by several modalities than trigeminal units in the thalamic nucleus ventralis posteromedialis (LANDGREN 1961).

As a tentative working hypothesis it is assumed that the posterior group of nuclei receives input both from ventral pathways and from spinal pathways relaying through the medial lemniscus. According to this hypothesis S II receives a dual somatic projection: 1) via the thalamic ventral basal complex to all S II and 2) via the posterior group of thalamic nuclei to the posterior part of S II.

Summary

In the studies of the cortical projections via the dorsal columns and via the lateral tract the majority of penetrations showed evoked slow wave potentials and unit activation only from the contralateral fore- or hindlimbs. In other penetrations in the posterior part of S II wave potentials and unit activation were obtained from large areas. It is assumed that the different distributions of the cortical effects evoked from small areas and from large areas is due to projection from two different thalamic relays. Fibres mediating the effect from small peripheral areas relay via the medial lemniscus in the thalamic ventral basal complex and project to S I and S II. Effects from large peripheral fields are assumed to relay in other nuclei, presumably the posterior group of thalamic nuclei. It is suggested that these nuclei receive effects from the specific projection pathways as well as from tracts ascending in the ventral spinal cord.

1959 ADEY and KERR 1954) and with single unit recording (MOUNTCASTLE 1957 MOUNTCASTLE and POWELL 1959 a CARRERAS and ANDERSSON 1962) In this investigation units were activated from deep structures via both the dorsal columns and the lateral tract No unit was activated by gentle movement of a joint but units were frequently obtained that were activated by light stimuli to other structures below the skin The receptive fields of these units were small Other units activated from deep structures had a high threshold and were activated from the vicinity of a joint or by muscle pressure Several units were activated from muscles and the majority were found in preparations with the lateral tract intact MOUNTCASTLE *et al* (1952) have given evidence that Group III muscle afferents project to the cerebral cortex In this investigation the cortical effects were obtained from pressure on the muscles and not from stretch which indicates that the effect is mediated by Group III rather than Group I or II muscle afferents (cf Discussion chapter VI) With regard to vibratory sensation clinical evidence in man suggests that this sense is transmitted by the lateral columns (NETSKY 1953) and dorsal columns (FOX and KLEMPERER 1942) In the present experiments cortical units were activated by vibratory stimuli via both the dorsal columns and the lateral tract

The question whether the dorsal column pathway and MORIN s pathway project to the same or to different populations of cortical cells has not been settled A study of this problem is in progress

In the present investigation on anaesthetized cats no evidence was found for short latency activation of S II via direct spino cerebellar pathways

The cortical units activated from large receptive fields or units with convergence of different modalities present special problems In experiments with intact spinal cord such units are found in S I (MOUNTCASTLE and POWELL 1959 a) but are more common in S II (CARRERAS and ANDERSSON 1962) The present study indicates that both the dorsal columns and the lateral tract can mediate unit activation in S II from large receptive fields (cf chapters III and IV) These units have similar properties to those found by WHITLOCK and PERL (1959) and by POGGIO and MOUNTCASTLE (1960) in the posterior group of thalamic nuclei and regarded as typical for the anterolateral system The hypothesis is here put forward that these effects do not project to S II via the ventral basal complex but rather via other thalamic nuclei presumably the posterior group of nuclei and further mainly to a special area of S II in the posterior part of the anterior ectosylvian gyrus

Cortical projection of pathways other than those with specific projection has been elucidated only indirectly in the present investigation

important function in tactile spatial discrimination The finding of afferent inhibition in the dorsal column pathway strongly supports this hypothesis with regard to the cat There is no evidence for MORIN's pathway in man (VERHAART 1957) and the evidence is conflicting regarding the existence of a lateral cervical nucleus in man (REXED 1952 GARDNER and MORIN 1957 CHAMBERS and LIU 1961) although both the nucleus and the pathway have been demonstrated in monkey (GARDNER and MORIN 1957) Therefore comparison of human and feline nervous systems should be made only with caution

The importance of afferent inhibition in spatial tactile discrimination is supported by its occurrence in other sensory systems and by the demonstration that such inhibition is an important factor in discrimination in these systems cf for example visual HARTLINE (1949) GRANIT (1955) JUNG (1961) RATLIFF (1961) auditory VON BÉKÉSY and ROSENBLITH (1951) KATSUKI (1961) However the small receptive fields for units activated via MORIN's pathway suggest that also this pathway might subserve spatial discrimination

There is as yet little information available concerning the function of spinal projection pathways in the intact animal In some experiments tactile placing reactions have been used as an index These reflexes remained after section of the dorsal columns (TOWER 1935 LUNDBERG and NORRSELL 1960) However the latter authors found that a unilateral lesion interrupting the lateral tract abolished the tactile placing reaction on the same side in cat and the suggestion was made that this effect was due to section of MORIN's pathway On the other hand later experiments with section of this pathway in the ventral funiculus rostral to its crossing to the opposite side did not lend support to this hypothesis (LUNDBERG 1962 personal communication) NORRSELL (1962) behaviourally conditioned dogs to puffs of air applied to the hindlimbs Transection of the dorsal columns in the lower thoracic cord did not change the conditioned reflex However following a unilateral interruption of the lateral tract in the dorsomedial part of the lateral funiculus no conditioned response could be evoked from the hindlimb on that side

The occurrence of inhibition in the dorsal column pathway probably decreases its synaptic security MORIN's pathway might be of special importance in mediating information about tactile events quickly and with a high degree of safety and it might possibly constitute the afferent link in spino cortico spinal reflexes

The projection from deep structures to S I and S II has a similar topographical pattern to the projection from the skin as evidenced both with the evoked potential technique (MOUNTCASTLE COVIAN and HARRISON

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In preparations with the spinal cord intact except for one of the specific projection pathways units with large receptive fields were obtained more frequently than in preparations in which only one of the specific projection pathways was intact and other parts of the cord transected. This suggests that transmission in the spinal cord outside the specific projection pathways is capable of activating cortical units. Effects on some of the units with large *receptive fields might represent cortical projection* from the spino-thalamic tract (GETZ 1952, MEHLER 1957, ANDERSSON and BERRY 1959). However, this problem could not be solved by selective *spinal cord lesions. The cortical effects elicited via the ventral spinal* cord after section of the specific projection pathways were quite different from those obtained through the specific projection pathways and in preparations with intact spinal cord. The cortical effects elicited via the ventral spinal pathways were widespread and found also outside the somatic sensory cortex. The explanation for this change in activation pattern is probably twofold. Section of descending pathways (located in the dorsal cord) tonically inhibiting the transmission to ascending ventral pathways results in these ascending pathways being much more effectively activated from the periphery (HOLMQVIST *et al.* 1960). In addition, section of the specific projection pathways gives a localized release of slow wave and grouped unit activity in the somato-sensory cortex, postulated to be due to the release of inhibition from these specific projection pathways upon a pacemaker mechanism activated via ventral ascending pathways (ANDERSSON 1962 a). For these reasons it is not possible to investigate the extent to which ventral pathways (spino-thalamic or spino-bulbar) contribute to cortical activation except by indirect methods.

The functional significance of the units activated from large receptive fields is difficult to evaluate. POGGIO and MOUNTCASTLE (1960) suggested *that the projection system activated from large fields and with con*vergence of different modalities might possibly subserve the sensibility of pain. In the present investigation many of the units with large receptive fields were most effectively activated by nociceptive stimuli and the possibility that these units are related to the sensation of pain must be considered. The finding that cortical units with large fields are activated *via different spinal pathways* is interesting in view of the diffuse spinal projection of pain in cat (KENNARD 1954).

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THE ALTERATIONS OF PLATELETS IN HEMOSTASIS

The function of blood platelets in hemostasis was shown by BIZZOZERO (1882), HAYEM (1882) and EBERTH & SCHIMMELBUSCH (1886) to depend on their ability to form a plug of cells which adheres to the break in the vessel wall and closes the defect The importance of this platelet plug has been repeatedly confirmed in later investigations (APITZ 1942 ZUCKER 1947, CHEN & TSAI 1948 HUGUES 1953) It may be concluded that hemostasis depends absolutely on the formation of this structure

Clearly the formation of a platelet plug which can resist the blood pressure demands strong adhesiveness between the elements of which it is composed and a strong attachment to the vessel wall Such adhesiveness is conspicuously absent from circulating platelets Neither is it observed in platelets suspended in plasma *in vitro*, if coagulation is prevented Evidently the platelets must undergo alterations by which they are rendered adhesive in order to become active in hemostasis This alteration was designated 'viscous metamorphosis' by EBERTH & SCHIMMELBUSCH (1886), a descriptive term indicating the change in platelets from non-

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THE ALTERATIONS OF PLATELETS DURING COAGULATION

Platelets undergo distinctive morphological alterations during the coagulation of blood These were accurately described by the pioneer investigators (HAYEM 1878, BIZZOZERO 1882, SCHIMMELBUSCH 1885) and more recently by STUBEL (1914), FERGUSON (1934), BEST, COWAN & MACLEAN (1938), FONIO (1940) and others Prominent among the platelet changes is the appearance of extreme adhesiveness, leading to the formation of platelet aggregates with adhesive properties towards other surfaces Simultaneously structural changes take place in the cells, which become more highly refractile and extend fine cytoplasmic fibrils, thereby becoming irregularly star-shaped Within the aggregates gradual disappearance of the cellular limits is observed, usually considered an expression of lysis of the cells

There is a striking similarity between these changes, occurring *in vitro* during coagulation, and those observed *in vivo* during the formation of the platelet plug In early theory, and in fact until recent years, this was explained by assuming the breakdown of platelets to initiate clotting (BIZZOZERO 1882, MORAWITZ 1905) In direct opposition to this view, however, WRIGHT & MINOT (1917) demonstrated that the morphological alterations of the platelets fail to appear when clotting is prevented by decalcification and promptly develop when the plasma is recalcified The authors concluded that the structural changes represent an effect upon the cells of factors formed in the coagulation process, an interpretation which is in complete agreement with recent studies of these phenomena

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Considerable uncertainty has existed concerning the nature of the clotting factor which produces viscous metamorphosis in plate

adhesive into adhesive elements *in vivo* It will be mentioned later that the same term is used differently in current literature

The cause and nature of this alteration of the platelets, and particularly its connection with clotting, represent fundamental problems in hemostasis APITZ (1939), from his studies of profibrin, suggested that platelets become adhesive through adsorption of this fibrin precursor Studies of the same author (APITZ 1942) and of ZUCKER (1947) demonstrate, however, that visible fibrin is absent in the early stages of the platelet plug Furthermore, hemostasis, measured by the bleeding time, usually proceeds normally in afibrinogenemia (PINNIGER & PRUNTY 1946, FRICK & McQUARRIE 1954, ALEXANDER *et al* 1954) It is therefore unlikely that fibrin formation is responsible for platelet adhesiveness HELLEM (1960) has demonstrated a factor in red blood cells (factor R) which initiates adhesiveness in platelets *in vitro* without the involvement of clotting According to HELLEM, BORCHGREVINK & AMES (1961) transfusions of red cells normalize the prolonged bleeding time which may be observed in anemic patients Whether this is due to the liberation of factor R from the erythrocytes appears, however, uncertain On the other hand, primary hemostasis is remarkably independent of plasma coagulation Thus in all congenital defects of clotting the bleeding time is normal In humans, treatment with heparin, dicumarol or related drugs has only a slight effect upon the primary bleeding time (BORCHGREVINK & WAALER 1958, and others) Animal experiments demonstrate that the prevention of platelet plug formation requires excessive concentrations of heparin, while lower amounts apparently stimulate the process (SOLANDT & BEST 1940, APITZ 1942, ZUCKER 1947, HUGUES 1953, FULTON *et al* 1953), although the plugs formed under these conditions may be hemostatically ineffective, a point which will be further discussed later It appears from these results that platelets acquire adhesiveness *in vivo* independently of the clotting process Yet, in experiments *in vitro*, only coagulation is able to produce irreversible aggregation of these cells

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THE ROLE OF *VISCOUS METAMORPHOSIS* IN THE FORMATION OF THE PLATELET PLUG

According to classical theory viscous metamorphosis occurs spontaneously in blood platelets when these are stimulated by contact with foreign surfaces or damaged endothelium Coagulation was believed to be *initiated by the breakdown of the metamorphosed* cells The explanation obviously became untenable as soon as it was clearly established that viscous metamorphosis is an effect, and not the cause, of coagulation Clotting must then be assumed to precede the platelet changes For reasons already discussed there have been objections, however, against the incorporation of the clotting process in the mechanism of the formation of the platelet plug Primary hemostasis can apparently take place without the *participation of coagulation* It thus seems necessary to assume the existence of a different type of adhesiveness, which is not produced by thrombin, to account for the collection of platelets into a hemostatic plug

A way to explain the paradoxical dissociation between blood *coagulation and primary hemostasis* has recently been pointed out by OWREN (1960) and BORCHGREVINK & OWREN (1961) From experiments on the hemostatic effect of transfusion of normal platelets to patients with hemophilia and factor V deficiency these investigators concluded that formation of thrombin is necessary in primary hemostasis It takes place, however, from coagulation factors carried by the platelets and is therefore largely independent of plasma clotting This removes all objections against including

coagulation in the processes of primary hemostasis, since it is explained why 'coagulability' of the platelets may be virtually independent of plasma clotting

It is in agreement with this interpretation, which rests on the existence of a platelet coagulation system capable of forming thrombin for viscous metamorphosis, that experiments on the formation of hemostatic platelet plugs point directly to the participation of viscous metamorphosis in the process APITZ (1942) observed that platelet plugs formed under strong, but not excessive heparinization were functionally ineffective because penetrable by the blood This indicated that consolidation of the platelet plug must be a necessary step in its development, and that this is an effect of thrombin on the cells ZUCKER (1947) similarly found that platelet plugs formed under strong heparinization and after intensive administration of dicumarol were hemostatically ineffective HUGUES (1953) studied the formation of platelet plugs under external irrigation of the lesion in the vessel Irrigation fluids containing 0 5 to 1 % sodium citrate or 0 25 % heparin prevented hemostasis, because the plugs formed were penetrable From these studies, and from observations on the effect of certain antihistaminic drugs known to inhibit viscous metamorphosis *in vitro* HUGUES (1959 b) has proposed that hemostatic plugs are formed in a three stage reaction whose elements are the following

1 An initial aggregation, with adhesion to the wound lips.
2 Further accumulation into a *platelet mass*, still penetrable by blood
3 Consolidation of the platelet mass to a hemostatically effective platelet plug

In the discussion of this scheme HUGUES does not exclude that stage 1 may be due to thrombin It takes place *rapidly*, in fact within a few seconds (HUGUES 1959 a), but may be suppressed by sufficiently high concentrations of anticoagulants Stages 2 and 3 are indisputable effects of thrombin and are interpreted as viscous metamorphosis and clotting of fibrinogen respectively Since it is generally agreed that fibrin *is not* an obligatory element of the platelet plug, the mechanism proposed by HUGUES (1959 b) may be considered to have two stages, the last stage being represented by viscous metamorphosis As far as is known all observations on

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same type of primitive contractile protein has a specific and different function in undifferentiated cells, where it was first demonstrated by HOFFMANN-BERLING (1956) In these cells the rounding up of the cytoplasm and subsequent equatorial contraction during cellular division are effected by the contractile protein Assuming that contractions are necessary for the formation of platelets from megacaryocytes, the presence of contractile protein in the mature platelet could be a vestigial phenomenon, related to the mechanism of cytoplasmic fragmentation of the mother cell

It is suggested in the studies of BETTEX GALLAND & LÜSCHER (1960, 1961) that the contractile protein of the platelets is responsible for the morphological alterations of viscous metamorphosis Furthermore, it is proposed that the contractile protein participates in clot retraction, either because it is secreted from the cells and combines with the fibrin fibres or because it is liberated with other cell constituents in a complex which is able to form contractile, pseudopodia like structures These would become attached to the fibrin fibres and contract the clot, in the manner previously described by BUDTZ OLSEN s theory (1951)

These hypotheses are mere hints towards a theoretical explanation of the functions of the contractile protein in platelets This, however, is a problem of second order, as long as it remains to be established that contractions actually occur The central question is whether contraction of platelets is initiated by exposure of the cells to thrombin

In an attempt to answer this question BETTEX GALLAND & LÜSCHER (1960) have studied the metabolism of blood platelets during their reaction with thrombin Marked changes were found, consisting in acute depression of respiration and a transient increase in glycolysis after addition of the enzyme This was followed by gradual extinction of all metabolic activity There was evidence of the simultaneous synthesis and breakdown of adenosine triphosphate (ATP) in the cells An initial gain in ATP was observed in the presence of glucose, followed by a breakdown to about 50 % of the original concentration Without glucose only ATP-degradation could be demonstrated An apparent parallelism was observed between the velocity and degree of clot retraction and the intensity of glycolysis and synthesis of ATP in the cells

the formation of platelet plugs and their function in hemostasis may be explained on this basis

It is therefore accepted in this work that viscous metamorphosis initiated by thrombin occurs *in vivo* and is essential for effective hemostasis, because it enables consolidation of the platelet plug. It is possible that the extreme adhesiveness of metamorphosing platelets also contributes during the building up of the platelet mass, but more doubtful whether it is responsible for the initial deposition of platelets

VISCOUS METAMORPHOSIS AND THE MECHANISM OF CLOT RETRACTION

Among the numerous attempts to explain clot retraction only one theory will be considered in the present work. It is the explanation given by BUDTZ-OLSEN (1951) in conclusion of his fundamental investigations. According to this theory clot retraction is an effect of contraction of fibrillar cytoplasmic extensions formed by the blood platelets in viscous metamorphosis. Mutual adhesions between the fibrils, and adhesions to the fibrin net, allow the shortening of these platelet extensions to produce clot retraction

It is necessary to refer to the original work for the extensive evidence on which BUDTZ OLSEN based this theory, which in the present studies has been accepted as proved. Some questions, however, are left unanswered. Thus the theory does not explain the actual mechanism of viscous metamorphosis and the appearance of cytoplasmic fibrils in this process. The nature of the contraction process in the platelet fibrils is also unknown

BETTEX-GALLAND & LUSCHER (1959, 1961) have recently rendered important contributions towards a deeper understanding of viscous metamorphosis and clot retraction by their discovery of an actomysin-like, contractile protein in blood platelets. The presence of this element in the cells suggests that contraction may be a fundamental element in their behaviour and properties. It cannot be taken for granted, however, that viscous metamorphosis and clot retraction are expressions of platelet contraction, until it is satisfactorily explained how the contractile protein may produce these effects. This appears of particular importance because the

Thrombin and platelets together form an enzyme substrate system of an unusual type, which may be expected to have properties which depart from proteolytic reactions in general Instead of being exposed to the enzyme in free solution, the substrate forms part of the platelet surface It cannot be predicted how its interaction with the enzyme may be influenced by this localization and by the configuration imposed upon the substrate by its incorporation in the structure of the platelet surface Furthermore, the morphological observations indicate that the alterations of viscous metamorphosis are more than a simple surface-effect and thus involve deeper structures in the cells It is not clear how these may be affected by a proteolytic reaction in the platelet surface

No elucidation of these relationships can be expected unless the processes are studied quantitatively However, viscous metamorphosis has been little explored from this point of view An attempt in this direction has been made by LÜSCHER (1956a) who employed clot retraction for the registration of viscous metamorphosis. It will be immediately recognized that this phenomenon only indirectly expresses the action of thrombin on platelets, since it depends upon the shortening of previously extended platelet fibrils (BUDTZ OLSEN 1951) Even if the formation of these fibrils should be conditioned by a reaction catalyzed by thrombin, it is doubtful how their subsequent shortening may be related to the velocity and extent of the preceding enzymatic process Clot retraction is therefore not suited for the measurement of the action of thrombin on platelets

A different effect exists, however, which is closely linked to the interaction of thrombin with platelets and which is suited for quantitative registration This is the thrombin-catalyzed release of 5-hydroxytryptamine (Serotonin) Admittedly it is unknown how this phenomenon may be related to viscous metamorphosis On the other hand, it is probably connected with the latter process. The following facts point in this direction

Thrombin causes a rapid release of 5-hydroxytryptamine from blood platelets, by a direct effect on the cells which is independent of the presence of other clotting factors (BIGELOW 1954, HUMPHREY & JAQUES 1955, ZUCKER & BORRELLI 1955, GRETTE 1959) Like viscous metamorphosis, release of 5-hydroxytryptamine is thus a specific effect of thrombin, dependent on the presence of a substrate for this enzyme in the platelet surface Thrombin is an

BETTEX-GALLAND & LUSCHER (1960) do not claim to have thus established that contraction occurs, although the observations are considered to point in this direction. In fact the results show that no answer can be obtained with regard to this question from studies of metabolism. Although splitting of ATP is generally accepted as the source of energy in muscular contraction, an undetermined part of this breakdown in platelets may represent the action of unspecific ATP ases. Furthermore, the kinetics and extent of breakdown cannot be determined, because of the simultaneous synthesis of ATP. Although the increase in glycolysis coincides with clot retraction, its contribution to this process appears unlikely, since there is no evidence that its product, in the form of ATP, is a limiting factor in retraction. About 50 % of the original ATP of the cells is still present when the process is completed. In *this connection it is noteworthy, although difficult to explain,* that the degree of breakdown of ATP was found to be higher in the presence of glucose than in its absence. Actually, glucose has *at most a weak effect on clot retraction. A 10 to 20 % stimulation* was observed by BETTEX-GALLAND & LUSCHER (1960) when aged platelets were used. No effect was obtained by ZUCKER & BORRELLI *(1959) or by* CORN, JACKSON & CONLEY *(1959). Apparently,* previous observations on the effect of glucose (LUSCHER 1956 b, 1956 c) were confused by pH-effects in unbuffered solutions. The *lack of clear correlations between the metabolism of platelets and* clot retraction is also evident in the studies of LOHR & WALLER (1959) on ATP and ability to produce clot retraction in platelets during ageing. *Both decrease during storage, but hardly in parallel.* Thus, after 14 days, 44 % of normal retraction was found in the presence of 3.5 % of the original ATP of the cells.

It is to be concluded that the occurrence of contraction in platelets exposed to thrombin, and its participation in viscous metamorphosis and clot retraction, remain to be demonstrated.

PROBLEMS CONCERNING THE MEASUREMENT OF THROMBIN-EFFECT AND VISCOUS METAMORPHOSIS IN BLOOD PLATELETS

Studies have been referred to above which demonstrate that viscous metamorphosis is an effect of thrombin on platelets. The *reaction mechanisms of this process are unknown, and it must be* considered how they may be subjected to further analysis.

Thrombin and platelets together form an enzyme substrate system of an unusual type, which may be expected to have properties which depart from proteolytic reactions in general Instead of being exposed to the enzyme in free solution, the substrate forms part of the platelet surface It cannot be predicted how its interaction with the enzyme may be influenced by this localization and by the configuration imposed upon the substrate by its incorporation *in the structure of* the platelet surface Furthermore, the morphological observations indicate that the alterations of viscous metamorphosis are more than a simple surface-effect and thus involve deeper structures in the cells It is not clear how these may be affected by a proteolytic reaction in the platelet surface

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extremely specific proteolytic enzyme, for which reason it is unlikely that there are two different constituents of the platelet surface which both have the property of acting as substrate for the enzyme It can be assumed, therefore, that viscous metamorphosis and release of 5-hydroxytryptamine are initiated by a single enzymatic reaction Furthermore, release of the intracellular constituent 5-hydroxytryptamine undoubtedly requires an alteration in the structure of the cells The nature of the release phenomenon is therefore not far removed from the manifestly structural changes of viscous metamorphosis

From the point of view of the present studies the release of 5-hydroxytryptamine is of theoretical and practical importance because of these connections with viscous metamorphosis If these phenomena are dependent on a common process in the platelets, its mechanism may be more easily studied by means of the release of 5-hydroxytryptamine, which is measurable by chemical methods

THE PRESENT STUDIES

The aims of the present investigation have already been defined as *concerning the mechanisms which are active in blood platelets* exposed to thrombin In the review of previous studies some questions were pointed out which appear to require further elucidation in this connection To the investigator it seemed of importance to attempt the identification of the substrate for thrombin in the platelets, as a preparatory step towards the study of the connections between hydrolysis of the thrombin-substrate, release of 5-hydroxytryptamine and viscous metamorphosis

Previous observations, to be referred to in detail later, have indicated the presence of fibrinogen in blood platelets This protein, whose sensitivity to the proteolytic effect of thrombin is manifested in clotting, was considered to be of special interest as a possible substrate in the reaction of the enzyme with the cells Its quantitative preparation and determination was accordingly attempted In the ensuing work on fractionation of platelet proteins the contractile protein present in these cells was identified The fundamental properties of this platelet constituent will be described, in confirmation of the earlier reports by BETTEX-GALLAND & LÜSCHER (1959, 1961) on its characteristics

It is not immediately obvious that the contractile protein of platelets is involved in the reaction of the cells with thrombin It has been mentioned that studies carried out on this question have yielded equivocal results And there seems to be no element of the platelet reaction which bears the unmistakable mark of contraction On the other hand, this impression is certainly based on insufficient knowledge Evidence of contraction may appear when the reaction of platelets with thrombin is studied in detail

It was decided to investigate the release of 5 hydroxytryptamine from this point of view, both with regard to the possible function of fibrinogen as substrate for thrombin and for possible signs of the occurrence of contraction Furthermore, it was planned to study the relations between release of 5 hydroxytryptamine and viscous metamorphosis and seek to establish whether these are equivalent effects of thrombin A question of importance is whether 5 hydroxytryptamine is unique in being released from the platelets, since information on this point may serve to indicate the nature of the thrombin effect Studies aimed at this problem form the first part of the investigation

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2 0 mg per ml) in 0 9 % NaCl buffered with 0 03 M Tris pH 7 5 The solutions were prepared immediately before use and were kept in siliconized test tubes A single lot of thrombin was employed for the experiments Its activity, determined on bovine fibrinogen as previously described (GRETTE 1959), was 8 8 thrombin units per mg No loss of potency was observed during several years of storage

TRIS BUFFER 0 15 M pH 7 5 Tris (hydroxymethyl) amino methane (Sigma) was dissolved in water, adjusted to pH 7 5 with HCl and diluted to volume One volume of this solution was added to 4 volumes of 0 9 % NaCl to give the buffered saline solution employed for suspending platelets Reagents to be added to platelet suspensions were made up with the same buffered saline

REAGENTS AND CHEMICALS Crystalline trypsin was obtained from Novo Terapeutisk Laboratorium Copenhagen, Mersalyl from Nyegaard & Co, Oslo, p chloromercuribenzoate (PCMB) and adenosine triphosphate (ATP), 99–100 % pure, from Sigma, disodium ethylenediaminetetraacetate (EDTA) from E Merck, Darmstadt All other chemicals were commercial *preparations* of analytical grade Solutions were made up with glass distilled water

INCUBATION OF PLATELETS AND DETERMINATION OF RELEASE Platelets suspended in 0 9 % NaCl buffered with Tris to pH 7 5 were incubated with thrombin in 5 ml glass centrifuge tubes in a water bath as described under the different experiments Controls were incubated in parallel without thrombin Incubation was terminated by centrifugation of the tubes at 4° C in an International PR 2 refrigerated centrifuge with multispeed attachment, $6000 \times g$ for 2 min (9200 r p m) Aliquots of the platelet free supernatant solutions were removed immediately and deproteinized with 3 volumes of 10 % trichloracetic acid (TCA) The deproteinized solutions were extracted three times by shaking with 3 volumes of water saturated, peroxyde free ethyl ether in test tubes closed with plastic stoppers in order to remove TCA Released adenine nucleotides were determined by measuring the optical density of the deproteinized and ether extracted samples at 259 mμ against blanks treated identically

DETERMINATION OF ATP ASE ACTIVITY 0 5 to 1 0 mg of contractile protein, in a final volume of 1 0 ml, was incubated at 22° C with ATP in a concentration of 5×10^{-4}M The medium con

CHAPTER I

Material and Methods

BLOOD PLATELETS Pig blood platelets were employed in all experiments in the present work The blood was collected directly into 1-litre bottles containing 150 ml of 3 14 % sodium citrate dihydrate, care being taken to obtain rapid and careful mixing with avoidance of foaming Occasionally, platelets were damaged during collection, as shown by their failure to withstand the subsequent isolation and washing This could not be prevented by siliconizing the bottles

The citrated blood was centrifuged at room temperature in 250 ml bottles in an International Size 2 centrifuge, 600 × *g* for 12 min (1700 r p m) The motor brushes were disconnected during deceleration The platelet-rich plasma was carefully siphoned off from the sedimented red cells and cooled to 4° C It was centrifuged at this temperature in 25 ml Lucite cups in an International PR 2 refrigerated centrifuge with multispeed attachment, 5000 × *g* for 4 min (8500 r p m) to sediment the platelets The platelets were washed three times in cold 0 9 % NaCl containing 0 1 volume of 3 14 % sodium citrate dihydrate

The yield of platelets sedimented after the last washing was determined by weighing and the cells were resuspended to a known concentration in cold 0 9 % NaCl buffered with 0 03 M Tris pH 7 5 The usual concentration was 0 10 g wet platelets per ml, which is about 15 times the concentration of platelets in citrated plasma

The intensity of the reaction of platelets with thrombin decreases during storage, especially when washed platelets are kept in saline suspension The citrated blood was therefore used within 2-4 hours, and platelet suspensions were prepared rapidly, in close connection with the experiments

THROMBIN Lyophilized bovine thrombin (Parke, Davis & Co) was weighed and dissolved to the desired concentration (0 5 to

2 0 mg per ml) in 0 9 % NaCl buffered with 0 03 M Tris pH 7 5 The solutions were prepared immediately before use and were kept in siliconized test tubes A single lot of thrombin was employed for the experiments Its activity determined on bovine fibrinogen as previously described (GRETTE 1959), was 8 8 thrombin units per mg No loss of potency was observed during several years of storage

TRIS BUFFER 0 15 M PH 7 5 Tris (hydroxymethyl) amino methane (Sigma) was dissolved in water, adjusted to pH 7 5 with HCl and diluted to volume One volume of this solution was added to 4 volumes of 0 9 % NaCl to give the buffered saline solution employed for suspending platelets Reagents to be added to platelet suspensions were made up with the same buffered saline

REAGENTS AND CHEMICALS Crystalline trypsin was obtained from Novo Terapeutisk Laboratorium Copenhagen, Mersalyl from Nyegaard & Co Oslo p chloromercuribenzoate (PCMB) and adenosine triphosphate (ATP), 99–100 % pure, from Sigma disodium ethylenediaminetetraacetate (EDTA) from E Merck, Darmstadt All other chemicals were commercial preparations of analytical grade Solutions were made up with glass distilled water

INCUBATION OF PLATELETS AND DETERMINATION OF RELEASE Platelets suspended in 0 9 % NaCl buffered with Tris to pH 7 5 were incubated with thrombin in 5 ml glass centrifuge tubes in a water bath as described under the different experiments Controls were incubated in parallel without thrombin Incubation was terminated by centrifugation of the tubes at 4° C in an International PR 2 refrigerated centrifuge with multispeed attachment 6000 × *g* for 2 min (9200 r p m) Aliquots of the platelet free supernatant solutions were removed immediately and deproteinized with 3 volumes of 10 % trichloracetic acid (TCA) The deproteinized solutions were extracted three times by shaking with 3 volumes of water saturated peroxyde free ethyl ether in test tubes closed with plastic stoppers in order to remove TCA Released adenine nucleotides were determined by measuring the optical density of the deproteinized and ether extracted samples at 259 mμ against blanks treated identically

DETERMINATION OF ATP ASE ACTIVITY 0 5 to 1 0 mg of contractile protein, in a final volume of 1 0 ml was incubated at 22° C with ATP in a concentration of 5×10^{-4}M The medium con

tained KCl 5×10^{-2}M, $MgSO_4$ 1×10^{-4}M and borate buffer pH 7 0 0 2M The enzymatic reaction was terminated by the addition of 1 ml of 10 % TCA The tubes were left at room temperature for 30 min and centrifuged Inorganic phosphate was determined in 1 0 ml of the deproteinized solution by addition of molybdate and extraction of phosphomolybdate into butanol according to MARSH (1959)

5-HYDROXYTRYPTAMINE was determined fluorimetrically by the method of UDENFRIEND, WEISSBACH & CLARK (1955) as described previously (GRETTE 1959)

CHROMATOGRAPHY OF PLATELET NUCLEOTIDES Perchloric acid extracts were analyzed on Dowex 1-formate columns as described by HURLBERT, SCHMITZ, BRUMM & POTTER (1954) Elution was performed with the gradients 0 to 4 M formic acid followed by 0 3 to 0 8 M ammonium-formate in 4 M formic acid The main fractions, representing AMP, ADP and ATP, were identified by their position on the chromatogram and their absorption spectra in acid solution, combined with determinations of total phosphate after wet ashing of 1 0 or 2 0 ml of sample The method of FISKE & SUBBAROW (1925) was employed for phosphate determination

TOTAL AMINO ACIDS (Ninhydrin-positive material) were determined by the method of YEMM & COCKING (1955) on 0 5 ml of sample The method was standardized with glycine

DETERMINATION OF RELEASED INORGANIC PHOSPHATE True and apparent inorganic phosphate released from platelets during incubation with thrombin was determined with the method of FISKE AND SUBBAROW (1925) Samples of 1 0 ml were employed

DETERMINATION OF INORGANIC PHOSPHATE FORMED BY ATP-ASE To avoid molybdate catalyzed hydrolysis of ATP, which may seriously affect the determination of ATP-ase activities, the method of MARSH (1959) was employed It was found necessary, however, to reduce the concentration of citrate to 0 1 of the original, in order to prevent decomposition of the phosphomolyb date complex by citrate In details the procedure was carried out as follows

1 0 ml of sample, containing 0 5 to 4 0 µg of inorganic phosphorus, was pipetted into a 15 ml round-bottomed centrifuge tube containing 5 0 ml of water and 4 0 ml of n-butanol 0 1 ml of a 4 % solution of sodium molybdate in 10 % HCl was added The tube was capped immediately with a plastic stopper and shaken

for 10 seconds 0 1 ml of a 4 % solution of trisodium citrate dihydrate was added and the tube was again shaken for 10 seconds It was then centrifuged briefly to separate the phases 0 1 ml of methanol was added and mixed into the butanol layer by cautious swirling, to remove cloudiness due to water droplets About 3 ml of the butanol was removed and read in the spectrophotometer at 310 mμ against a blank which contained all reagents, including TCA

DETERMINATION OF PROTEIN was carried out with the biuret method as described by GORNALL, BARDAWILL & DAVID (1949) For concentrations between 0 25 and 1 0 mg of protein per ml equal volumes of reagent and sample and at higher concentrations 4 volumes of reagent to 1 volume of sample, were employed The method was standardized with crystalline bovine serum albumin

ENZYMATIC DETERMINATION OF ATP IN PLATELETS The principle of this assay has been described by BUCHER (1955) for the determination of phosphoglycerate kinase activity (back reaction) With a surplus of enzymes and sufficiently high concentrations of 3 phosphoglycerate and DPNH the oxidation to DPN is quantitatively determined by the amount of ATP which is present The oxidation of DPNH is determined spectrophotometrically In the present experiments deproteinized and neutralized perchloric acid extracts of platelets were prepared as described The cuvette contained 1 0 ml of 6 × 10 - M 3 phosphoglycerate, 20 μl of 1 2 × 10 - M DPNH and 100 μl of platelet extract, to which 20 μl of enzyme suspension (phosphoglycerate kinase/glyceraldehydephosphate dehydrogenase) was added A decrease in optical density at 340 mμ of 0 100 corresponds to the presence of 0 018 μmoles of ATP Oxidation of DPNH was limited to 10 % of the initial concentration in the cuvette The reagents were obtained commercially from C F Boehringer & Soehne, Mannheim

DETERMINATION OF FIBRINOGEN One volume of the solution was clotted with 0 5 volume of thrombin in a water bath at 37° C The clot was compressed with a glass rod after 30 min, washed twice in 0 9 % NaCl blotted and dissolved directly in the biuret reagent

VISCOSIMETRY The measurements were carried out at 22° C An Ostwald viscosimeter with a volume of 5 ml and a flow time for water of 116 seconds was employed

INSTRUMENTS Photometric determinations were carried out

with a Hilger Biochem photometer and with a Beckman spectrophotometer model DU, which was equipped with diaphragm and 1 ml cuvettes Spectra were determined with the Beckman instrument For the determination of 5-hydroxytryptamine a Farrand Ultraviolet Spectrophotofluorimeter was employed pH measurements were made on direct reading instruments from Radiometer and Metrohm, standardized with buffers supplied by these firms

ABBREVIATIONS The following abbreviations are employed in the text AMP, ADP and ATP, the mono , di- and triphosphates of adenosine, DPN, DPNH, oxidized and reduced diphosphopyridine nucleotide, 5-HT, 5-hydroxytryptamine (Serotonin), EDTA, ethylenediamine tetraacetate, PCMB, p-chloromercuribenzoate, Tris, tris (hydroxymethyl) aminomethane, TCA, trichloracetic acid

CHAPTER 2

Observations on the Release Reaction Initiated in Platelets by Thrombin

It has already been pointed out that release of 5-hydroxytryptamine represents a specific action of thrombin on platelets, which it may be possible to follow quantitatively by determination of the amount of released material An example of this effect of thrombin, taken from a previous report (GRETTE 1959), is shown in Figure 1, and demonstrates some of the characteristics of the process

Release is seen to start immediately upon addition of thrombin to the platelet suspension The reaction proceeds with a very high initial velocity, and substantial amounts of 5-hydroxytryptamine are released within the first minute of incubation

This interference of thrombin with the ability of platelets to retain 5-hydroxytryptamine shows that a substrate for the enzyme

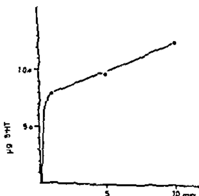


Fig 1 Release of 5 hydroxytryptamine from platelets by thrombin Samples of platelets (0 05 g) were incubated with 0 75 mg of thrombin in Tris buffered saline pH 7 5 at 37° C Total volume 1 5 ml The samples were centrifuged at times indicated on the abscissa and 5 hydroxytryptamine was determined in 1 ml of the platelet free supernatant solutions

with a Hilger Biochem photometer and with a Beckman spectro photometer model DU, which was equipped with diaphragm and 1 ml cuvettes Spectra were determined with the Beckman instru ment For the determination of 5-hydroxytryptamine a Farrand Ultraviolet Spectrophotofluorimeter was employed pH measure ments were made on direct reading instruments from Radiometer and Metrohm, standardized with buffers supplied by these firms

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strated appreciable concentrations of adenine nucleotides in blood platelets It was thus considered likely that the substances released are adenine nucleotides This was confirmed by chromatographic

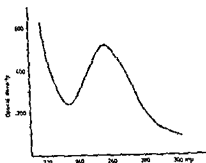


Fig 2 Absorption spectrum of the medium after incubation of platelets with thr [illegible]
Tr [illegible]
an [illegible]
TCA The deproteinized solution was extracted three times with 3 volumes of ethyl ether Optical density of the solution was determined at the different wavelengths against an identically treated blank

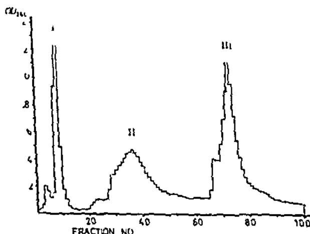


Fig 3 Chromatography of nucleotides released from platelets by thrombin Platelets (5 0 g) were incubated with 5 mg of thrombin in 25 ml Tris buffered saline pH 7 5 at 37° C for 15 min The suspension was centrifuged and 14 ml platelet free medium was deproteinized with 28 ml 0 6 N perchloric acid Chromatography of the deproteinized and neutralized solution was carried out on a 10 cm column of Dowex-1 formate Peaks I, II, and III were identified as AMP, ADP, and ATP

is present in the cells The immediate onset suggests that this substrate is freely accessible, which probably means that it is present in the platelet surface The known proteolytic nature of the enzyme thrombin indicates that *the substrate is a protein*

There is at present no information on the mechanism by which 5-hydroxytryptamine is held within the structure of the platelets, and it is therefore a matter of speculation how this mechanism is destroyed by the reaction between thrombin and its substrate in the cell To the further considerations it is a question of importance whether the action of thrombin in this respect is specific and limited to 5-hydroxytryptamine, or whether it is more extensive Release of only a single intracellular constituent indicates a localized interaction with a specific binding-mechanism for this substance On the other hand, *if various intracellular constituents* are released together, more extensive destruction of the cellular organization is suggested It was therefore necessary to investigate the possible release of other intracellular material in addition to 5-hydroxytryptamine

An answer to the question outlined above was obtained by measuring the absorption spectrum of the medium in which platelets had been incubated with thrombin *If only 5 hydroxy*-tryptamine is released no changes can be expected, since the amounts are too small to be demonstrated by direct spectrophotometry It was found, however, that other substances which possessed strong and characteristic absorption of ultraviolet light were present in the medium after the incubation with thrombin Evidently these substances had been released from the platelets

An example of the ultraviolet absorption of the medium, after removal of the incubated platelets and deproteinization, is shown in Figure 2 It is a highly characteristic curve, which shows an *absorption maximum at 259 mμ* and in details corresponds with the absorption curve of adenine

The result indicates, therefore, that adenine or adenine compounds are released from blood platelets in addition to 5 hydroxytryptamine during incubation with thrombin

With regard to the possible nature of the adenine compounds released from the platelets it is of interest that previous studies by other investigators (BESTETTI & CROSTI 1955, BORN 1956 a, FANTI & WARD 1956, MIZUNO, SAUTER & SCHULTZE 1960) have demon

strated appreciable concentrations of adenine nucleotides in blood platelets It was thus considered likely that the substances released are adenine nucleotides This was confirmed by chromatographic

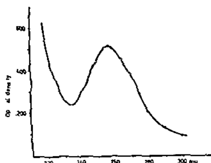
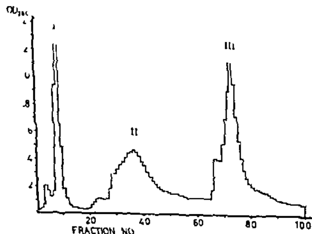


Fig 2 Absorption spectrum of the medium after incubation of platelets with thrombin Platelets (0 15 g) were incubated with 1 0 mg of thrombin in 1 5 ml Tris buffered saline pH 7 5 at 37° C for 5 min The suspension was centrifuged and 1 ml platelet free supernatant solution was deproteinized with 2 5 ml 10 % TCA The deproteinized solution was extracted three times with 3 volumes of ethyl ether Optical density of the solution was determined at the different wavelengths against an identically treated blank



Chromatography of the deproteinized and neutralized solution was carried out on a 10 cm column of Dowex 1 formate Peaks I II, and III were identified as AMP, ADP, and ATP ... N perchloric acid

analysis of material released from a large portion of platelets (Fig 3)

The substances present in the three fractions shown in Figure 3 gave pure adenine spectra in acid solution, and their positions on the chromatogram corresponded to those of AMP, ADP, and ATP The results of an analysis of an acid extract of whole plate lets, carried out with the same system, are shown in Figure 4 In this chromatogram the three main fractions were identified as AMP, ADP, and ATP from the spectral absorption curves com bined with determinations of total phosphorus

From these results it was concluded that the adenine compounds released from platelets by thrombin are a mixture of the mono , di and triphosphates of adenosine The total amounts of adenine *nucleotides in intact platelets were also determined The results of* the chromatographical determinations in three preparations of washed platelets are given in Table I

In accord with the above mentioned previous observations ATP is found to represent the major fraction of the nucleotides of intact platelets Relatively high concentrations of this substance are present in the cells

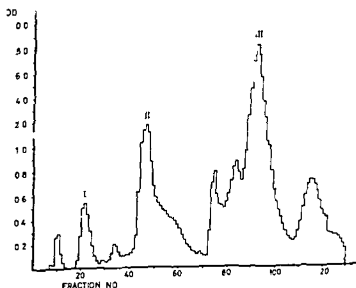


Fig 4 Chromatography of nucleotides extracted from whole platelets Platelets (5 7 g) were extracted with 55 ml 0 6 N perchloric acid The extract was neutralized and chromatographed on a 10 cm Dowex 1 formate column Peaks I, II, and III were identified as AMP ADP and ATP

Table I

ADENINE NUCLEOTIDES IN WASHED PLATELETS

	Adenine nucleotides μmoles/g platelets		
	AMP	ADP	ATP
Preparation 1	0 08	0 98	2 06
" 2	0 15	1 24	3 10
" 3	0 16	0 87	2 38

Three samples of washed platelets (3 0, 3 8, and 5 7 g) were extracted with 10 volumes of 0 6 N perchloric acid The extracts were neutralized and chromatographed on Dowex 1 formate AMP, ADP, and ATP in the three adenine nucleotide peaks were determined from the volumes of these fractions and the optical densities measured at 257 mμ against the elution fluid

The demonstrated release of adenine nucleotides together with 5 hydroxytryptamine suggests that the action of thrombin involves essential structures in the cells, with the unspecific result that there is a general liberation of intracellular constituents. This conclusion was verified by the following experiment, of which the results are shown in Figure 5

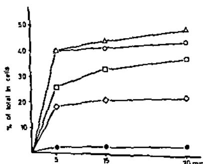


Figure 5 The parallel release of different intracellular constituents from platelets by thrombin Samples of platelets (0 25 g) were incubated with 1 0 mg of thrombin in 2 5 ml of Tris buffered saline pH 7 5 at 37° C and centrifuged at times indicated on the abscissa 2 ml of the supernatant solutions were deproteinized with TCA and extracted with ether 5 hydroxytryptamine △, adenine nucleotides ○, inorganic phosphate □, and free amino acids ◇ were determined in aliquots of the deproteinized solutions. Released protein precipitated with TCA was dissolved in 1 N NaOH and determined ⊕ The cells sedimented after incubation were extracted with TCA in order to determine their content of the same substances after exposure to thrombin The protein precipitated from these cells was dissolved in 1 N NaOH and determined From these results the percentage of release for each substance was calculated

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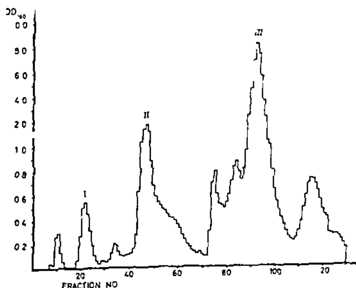


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SUMMARY OF CHAPTER 2

The releasing effect of thrombin on different intracellular constituents of platelets is described It is shown that, in addition to 5-hydroxytryptamine previously known to be released from platelets, a wide range of intracellular components of different characteristics (adenine nucleotides, free amino acids, inorganic phosphate, protein) are liberated when the cells are exposed to thrombin

These different constituents are liberated in parallel The extent of their release, however, differs markedly 5-hydroxytryptamine and adenine nucleotides are most extensively liberated, while only a few per cent of cellular protein is set free

It is concluded that the decrease in the ability of the cells to withhold intracellular constituents, which is produced by the action of thrombin, is an expression of a process which differs from simple lysis of the cells It is proposed to call this process the release reaction of the platelets

The closely parallel time course in the release of different platelet constituents demonstrates that their liberation is due to a common process in the cells Any of these substances may therefore be employed as markers in following the course of the release reaction Adenine nucleotides are especially suitable in this respect, since their quantitative determination can be carried out by *direct* spectrophotometry Studies on the kinetics of the release reaction, performed in the present work, were therefore based on the determination of released adenine nucleotides

In the experiment 5-hydroxytryptamine, adenine, ninhydrin positive material assumed to be mainly represented by amino acids, protein, and inorganic phosphate were simultaneously determined in the supernatant solution from a suspension of platelets incubated with thrombin It is seen from Figure 5 that these different substances, which have only the common characteristic of being *strictly intracellular* components, are all released

Such extensive liberation of substances normally confined within the cells may suggest the occurrence of platelet lysis It is questionable, however, whether this term adequately describes the thrombin-catalyzed process Cellular lysis is generally understood to define a destruction of cellular membranes which completely removes all permeability barriers and allows free passage of intracellular constituents into the medium The increase in permeability which depends upon the action of thrombin is of a different nature, since it is selective This is shown by the fact that different intracellular constituents are released from the platelets independently of their concentrations in the cells (Fig 5) The very small amounts of protein which are liberated form a striking example

In accord with these results it will be demonstrated (Chapter 6) that thrombin-catalyzed liberation of intracellular constituents from platelets takes place by a process which has no resemblance to simple lysis of the cells It is proposed to describe this phenomenon as the *release reaction of the platelets*, a term which will be used throughout this work

As shown above the time course of liberation is remarkably similar for the different constituents which take part in the release reaction Because of this parallelism it is a matter of convenience whether 5-hydroxytryptamine or a different platelet constituent is selected as indicator substance for studies on the progress of release For analytical reasons the estimation of adenine nucleotides is especially suitable, since the high specific absorption of these compounds allows their determination by direct spectrophotometry in the deproteinized medium, whereas determination of 5-hydroxytryptamine is a relatively complicated and time consuming procedure In all following studies in which release was measured the liberated adenine nucleotides were therefore employed for its registration

in the platelets It could not be determined by these investigators whether fibrinogen or a different protein was responsible for this property of platelet extracts Modern immunological techniques, which were applied to this problem by SALMON & BOUNAMEAUX (1956, 1957), and by SELIGMANN *et al* (1957), have demonstrated, however, that the coagulable material in platelets is actually fibrinogen LUSCHER (1959) has come to the same results by chromatography and ultracentrifugation of platelet extracts.

It is of importance to the considerations concerning the functional role of platelet fibrinogen whether this constituent merely consists of fibrinogen absorbed on the surface of the platelet or whether it is an integral component of the cell Both SALMON & BOUNAMEAUX (1956) and SELIGMANN *et al* (1957) employed platelets which had been washed extensively Yet they contained fibrinogen, which may suggest that it is present intracellularly A purified anti fibrinogen serum was found by SALMON & BOUNAMEAUX (1957) to be only weakly agglutinating towards washed platelets, although it reacted strongly with fibrinogen in platelet extracts This indicated that little or no fibrinogen is located superficially again suggesting that it is a cytoplasmic constituent On the other hand, in a patient with congenital afibrinogenemia studied by SALMON, VERSTRAETE & BOUNAMEAUX (1957) no fibrinogen was demonstrated immunologically in the platelets, possibly because adsorption had been prevented by the lack of fibrinogen in the plasma The suppression of fibrinogen formation in afibrinogenemia, however, must be expected to include its possible synthesis in the megacaryocytes The observation is therefore equivocal with respect to the question of the origin of fibrinogen in the platelets.

Only if it is truly a structural constituent of the cells can it be considered likely that platelet fibrinogen may function as the substrate for thrombin in the release reaction It is difficult to see how coagulation of superficially adsorbed fibrinogen, or rather the proteolytic alteration by which it is rendered coagulable, could be instrumental in this process. These considerations led to a search for further information on the localization of fibrinogen in the platelets Quantitative determination of platelet fibrinogen appeared to be of value in this connection

Evidently determination of fibrinogen in platelets demands

CHAPTER 3

Extraction and Partial Purification of Platelet-Fibrinogen

THE PRESENCE OF FIBRINOGEN IN PLATELETS

The characteristics of the release reaction, described in Chapter 2, indicate that the substrate on which thrombin is acting in the platelet is a part of the cellular structure on which the maintenance of its integrity is dependent The immediate and extremely rapid course of the release process points to a key role of the intact thrombin-substrate in the preservation of the cell

In order to elucidate further the mechanism of this remarkable process, by which a perfectly stable cellular structure is suddenly destroyed, *it was considered to be of interest to identify the substrate* or trigger-substance on which thrombin is acting The characteristic substrate specificity of this enzyme (SHERRY & TROLL 1954) is then of special importance, since it serves to limit the number of proteins which come into consideration as possible substrates Actually, fibrinogen is the only well characterized protein known to be rapidly hydrolyzed by thrombin, in a reaction which involves a limited number of peptide bonds at specific sites in the molecule (BAILEY & BETTELHEIM 1955, BETTELHEIM 1956, BLOMBÄCK & VESTERMARK 1958, and others) The high initial velocity of the release process indicates that the essential step, the *hydrolysis of the platelet substrate by thrombin, takes place with* comparable velocity

It may therefore be significant that fibrinogen is regularly found to be present in extracts of platelets, suggesting that it is a normal constituent of the cell The first observations in this direction were made by WARE, FAHEY & SEEGERS (1948), who reported that extracts of platelets coagulate when thrombin is added The studies of JOHNSON & SCHNEIDER (1953) and SCHNEIDER *et al* (1954) confirmed the presence of coagulable material

After 1 hour at 4° C the extract is centrifuged for 5 minutes at 7000 × g A large protein precipitate, with the appearance of a semitranslucent gel, is hereby sedimented The turbid supernatant solution is further fractionated

3 2ND PRECIPITATION Cold ethanol is added to the solution dropwise, with continuous stirring, to a final concentration of 5 % The mixture is held at about 3° C for 4 hours and is then centrifuged A small precipitate is removed

4 3RD PRECIPITATION The supernatant solution is diluted with 2 volumes of cold 0 015 M $MgSO_4$ Cold ethanol is added dropwise to a final concentration of 5 % and the mixture is left overnight at 4° C The precipitated fraction contains the fibrinogen of the platelets The sediment is collected by centrifugation and dissolved in 0 3 M KCL containing 0 015 M Tris buffer pH 7 5 to 1/3 of the volume of the original platelet extract Insoluble material is removed by ultracentrifugation (Spinco preparative ultracentrifuge, head SW 35, 80 000 × g for 30 min) The slightly turbid supernatant solution contains about 3 % of coagulable protein It is stable for 2–3 days in the refrigerator

THE FIBRINOGEN CONTENT OF THE PLATELETS

The fraction obtained by the procedure described above contains fibrinogen and forms a typical fibrin clot on addition of thrombin Contaminating proteins are present in the solution, however, as shown by its relatively low coagulability This does not influence the quantitative determination of fibrinogen, provided it is assured that the contaminants are not coprecipitated during clotting For this reason it is necessary to subject the final solution to ultracentrifugation before its protein content and coagulability is determined The importance of the latter point is illustrated by the results given in Table II, in which two of the samples (*preparations* 3 and 4) were not ultracentrifuged and therefore contained insoluble material

The loss of fibrinogen during fractionation of the platelet extract could not be determined, because of the occurrence of coprecipitation during clotting in all except the *final fractions* The figures shown in Table II, preparations 1 and 2, indicate that at least 3 5 4 % of the total soluble protein in platelets is fibrinogen

solubilization of the protein and removal of material which may coprecipitate during clotting The following method was developed to make this possible

EXTRACTION AND FRACTIONATION

When platelets are extracted with solutions of physiologic strength difficulties arise due to the low solubility of a large fraction of cellular protein Apparently this fraction is able to prevent complete extraction of platelet fibrinogen On the other hand, when present as a contaminant in the extracts this component may confuse the results by its coprecipitation with fibrin during clotting In order to bring the proteins of the platelets completely into solution it was found necessary to employ solutions of high ionic strength 0 6 M KCL was finally adopted as extractant

Platelets do not dissolve spontaneously in 0 6 M KCL solution and are difficult to break efficiently by the usual procedures of homogenization It was found, however, that addition of small amounts of n butanol effected almost immediate lysis of the cells which enabled the cellular proteins to be extracted From this extract a fraction containing platelet fibrinogen was precipitated by stepwise dilution and addition of ethanol The steps of this procedure are described below

1 EXTRACTION Platelets obtained from citrated pig blood are washed 3 times in citrated saline in the cold The cells sedimented from the last washing are weighed and 2 ml of cold 0 6 M KCL containing 0 015 M Tris buffer pH 7 5 are added per g of wet cells The cells are suspended and 25 μl of n butanol are added per ml of final solution Mixing is obtained by repeatedly drawing the suspension into a glass pipette with rubber bulb During these manipulations the material flocculates and then dissolves in the course of 2 3 minutes Extraction is allowed to proceed for 1 hour at 4° C

2 1ST PRECIPITATION The solution is warmed to room temperature and centrifuged without cooling for 4 minutes at 5000 g in the angle head Small amounts of insoluble material are removed To the supernatant solution an equal volume of cold 0 015 M $MgSO_4$ is added and pH is adjusted to 6 5 with 1 acetic acid

CHAPTER 4

The Action of Trypsin and Thrombin on Platelet-Fibrinogen

EXPOSURE OF PLATELETS TO TRYPSIN AND SUBSEQUENT LIBERATION OF PLATELET FIBRINOGEN WITH CALCIUM IONS

The results of quantitative determination of fibrinogen in platelets (Chapter 3) had to be considered as equivocal with respect to the localization of this protein within the cells A different approach was therefore necessary and it was decided to study the effect of trypsin

The literature contains only scattered references to the action of trypsin on blood platelets BUDTZ OLSEN (1951) studied viscous metamorphosis in recalcified platelet rich plasma to which trypsin had been added Digestion of the fibrin clot was observed to take place while viscous metamorphosis proceeded normally HUMPHREY & JAQUES (1955) observed rapid liberation of histamine from platelets with trypsin HJORT RAPAPORT & OWREN (1955) found that trypsin destroys platelet proaccelerin (factor V) without notable alterations in morphology, concluding that this clotting factor is adsorbed upon the platelet surface To judge from these results trypsin is unable to produce viscous metamorphosis in platelets The observations suggest that this enzyme has a superficial effect which is limited to proteins adsorbed upon the platelet surface

With this in view it was expected that treatment with trypsin would demonstrate whether fibrinogen is an intracellular constituent of blood platelets since it would then appear resistant to the enzyme On the other hand, fibrinogen adsorbed upon the platelet surface would probably be removed by trypsin digestion.

Experiments carried out on these assumptions, however, demonstrated that the action of trypsin on blood platelets is more

Table II

FIBRINOGEN IN WASHED PLATELETS

	Weight of cells	Total protein	Coagulable fraction	Coagulability	Content of fibrinogen	Fibrinogen in %
1	16 3 g	1550 mg	120 mg	41 %	49 mg	3 6
2	13 8 »	1200 »	107 »	46 »	49 »	4 1
3	12 8 »	1080 »	114 »	63 »	72 »	6 7
4	11 0 »	870 »	94 »	75 »	70 »	8 0

Platelets from citrated pig blood were washed extracted with 0 6 M KCl and the extract fractionated as described in the text The final fractions from preparations 1 and 2 were ultracentrifuged (80 000 × g for 30 min) This was omitted in preparations 3 and 4 Total protein was determined in aliquots of the crude extracts and the final fractions Fibrinogen was determined after clotting 0 4 ml of the latter fractions with thrombin

It was considered unlikely that these amounts of fibrinogen are able to stay adsorbed upon the surface of the platelets and resist the washing of the cells, although this possibility cannot be excluded The result therefore suggested, but could not prove, that fibrinogen is a component of the cytoplasm of the platelet

SUMMARY OF CHAPTER 3

Investigations of fibrinogen in blood platelets, carried out with a view to the possible role of this protein as substrate for thrombin *in the release reaction, are described It is pointed out as a funda* mental question in this connection whether fibrinogen is a true cytoplasmic constituent of blood platelets, or whether it only exists in passive adsorption upon the surface of the cells In spite of investigations aimed at this question, the state of fibrinogen in platelets is not known

With the object of being able to determine the amounts of fibrinogen in platelets a new method was developed for the extraction and fractionation of their constituent proteins With this method a partially purified *fibrinogen fraction* was prepared from washed platelets Determinations of the total protein and the coagulability of this fraction demonstrated that about 4 % of the protein of platelets is fibrinogen

It is provisionally concluded, from the fact that it is present in platelets to the extent of several per cent of their total protein, that fibrinogen may be an intracellular constituent of platelets

able to act on true platelet constituents An observed susceptibility towards this enzyme therefore does not establish that a given platelet component is present in an adsorbed state upon the surface of the cell On the other hand, a platelet constituent which is able to resist trypsin, although it is known to be hydrolyzed by the enzyme when in free solution, may be assumed to be located intra cellularly

By these criteria platelet fibrinogen was found to behave as a true intracellular constituent A platelet suspension incubated with trypsin and subsequently extracted by the method previously described (Chapter 3) was found to yield fibrinogen, indicating that this protein had been protected by its intracellular localization against the enzyme An additional way of demonstrating that platelet fibrinogen is refractory towards trypsin was also discovered This depends upon the observation that *trypsin treated and washed platelets carry out a release reaction when exposed to calcium ions*

In order to demonstrate this fact the trypsin treated cells

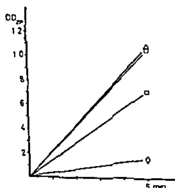


Fig Release of adenine nucleotides from trypsin treated cells by calcium. Samples of trypsin treated cells from the preceding experiment (Fig 6), sedimented by centrifugation after incubation with the enzyme for 5 to 20 min, were washed once in 4 ml of cold *Tris buffered* saline pH 7 5 and resuspended in 1 5 ml of this solution The smooth suspensions were warmed to 37° C (water bath 5 min) and 0 1 ml of 2 × 10 ² M Ca was added The tubes were incubated for 5 min and centrifuged 0 5 ml platelet free supernatant solution was deproteinized with TCA and analyzed for adenine nucleotides. The duration of trypsin treatment before recalcification is indicated by the different symbols 5 min with trypsin ○, 10 min △, 20 min □ Control incubated without trypsin ◇

complex than has been formerly believed, firstly, because an effect which is not limited to proteins adsorbed upon the cellular surface was indicated, and secondly, because platelets exposed to trypsin were found to acquire the property of subsequently reacting with calcium ions in a specific and fundamental manner

A structural effect of trypsin, demonstrating involvement of platelet constituents which are essential parts of the cellular organization, was evidenced by the occurrence of liberation of 5-hydroxytryptamine and adenine nucleotides during incubation (Fig 6)

This release of typical intracellular constituents appears very similar to the previously demonstrated effect of thrombin (Fig 5, Chapter 2), except for the fact that viscous metamorphosis was not produced Platelets sedimented by centrifugation after incubation with trypsin were freely suspendable in saline solutions

It is, however, without significance in this connection whether the action of trypsin on platelets is related to that of thrombin The experiment shown in Figure 6 demonstrates clearly that trypsin, in addition to its possible effect on adsorbed proteins, is

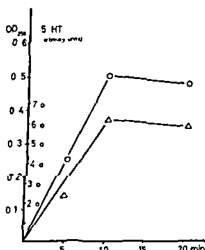


Fig 6 Release of 5 hydroxytryptamine and adenine nucleotides from platelets by trypsin Samples of platelets (0 2 g) were incubated with 0 2 mg of crystal line trypsin at pH 7 7 and 20° C in 0 15 M NaCl containing 0 1 volume of 3 14 % sodium citrate dihydrate Total volume 5 ml Controls were incubated without trypsin Centrifugation of the samples was carried out at times indicated on the abscissa Adenine nucleotides ○ and 5 hydroxytryptamine △ were determined in aliquots of the deproteinized platelet free supernatant solutions

Table III

RELEASE OF FIBRINOGEN
FROM TRYPSIN TREATED CELLS BY CALCIUM

Trypsin treatment	Protein released per g of platelets	Coagulability of released protein	Fibrinogen released per g of platelets
5 min	5 5 mg	45 %	2 5 mg
10 »	5 4 »	37 »	2 0 »
20 »	5 1 »	33 »	1 7 »

The experiment was carried out as described under Fig 7 with incubation of washed platelets with trypsin and subsequent recalcification The cells were removed by centrifugation Total protein and fibrinogen were determined in 0 5 ml aliquots of the platelet free supernatant solutions

Chapter 3) The participation of platelet-fibrinogen in this release reaction is therefore specific for this protein

It is seen (Table III) that less fibrinogen is released from the cells after prolonged treatment with trypsin This is not, however, an expression of proteolytic digestion of the protein As shown in Figures 7 and 8, prolonged incubation also decreases the subsequent release of 5 hydroxytryptamine and adenine nucleotides. This somewhat paradoxical result indicates that trypsin, in addition to its ability to alter the cells so that calcium may initiate release, also has a damaging effect on elements of the release mechanism

It is possible to conclude, therefore, that platelet fibrinogen is unaffected when the cells are incubated with trypsin, which indicates that this protein is a true intracellular constituent

With this information we may return to the original question which motivated the studies on platelet-fibrinogen in the present work namely whether this protein represents the substrate for thrombin in the release reaction Theoretically this may be possible, since platelet fibrinogen is a true platelet component, but it can only be certainly established by demonstrating that fibrinogen in platelets is attacked by thrombin in the course of the release reaction

DEMONSTRATION OF CORRESPONDENCE BETWEEN THE ACTION OF TRYPSIN AND OF THROMBIN IN THE INITIATION OF RELEASE

The experiments with trypsin (Figs 6–8) indicate that under these conditions release is produced by a two-stage process There is a primary reaction between the enzyme and the cells, in which

employed in the experiment of Figure 6 were washed once and then incubated for 5 min at 37° C with Ca ions in a final concentration of 5 mM. A substantial and rapid release of adenine nucleotides and 5-hydroxytryptamine occurred (Figs 7 and 8). During this incubation with calcium a rapid formation of coarse platelet aggregates, indistinguishable from true viscous metamorphosis, was observed. The latter phenomenon will be further discussed in Chapter 7.

From the present point of view the following additional observation is of importance. *The release initiated by trypsin and subsequent exposure to calcium ions liberates platelet fibrinogen.*

In the experiment already described (Figs 7 and 8) the released fibrinogen was demonstrated by addition of thrombin to aliquots of the platelet free supernatant solutions obtained after incubation with calcium and centrifugation. Well-defined clots were formed, and their protein contents were determined. Total protein of the solutions was simultaneously analyzed. The results are shown in Table III.

The experiment demonstrates that fibrinogen, and only small amounts of other proteins, are liberated in the release reaction initiated as described above. The coagulability of the released protein is relatively high, in fact comparable to that of the previously described preparations obtained by extraction (Table II,

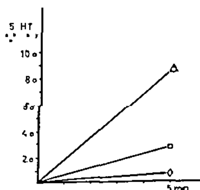


Fig 8 Release of 5 hydroxytryptamine from trypsin treated cells by calcium. The deproteinized ether extracted solutions obtained as described under Fig 7 and employed for the determination of adenine nucleotides were analyzed for 5 hydroxytryptamine. *Aliquots of 0.5 ml* were used. The symbols indicate the duration of trypsin treatment before recalcification: 5 min, 10 min, 20 min ⌜, control ›

plete accordance with the previous observations on trypsin treated cells the released fibrinogen coagulates spontaneously in 5–10 min at 37° C The clot undergoes retraction An experiment in which these phenomena were photographed is shown in Figure 9

It is shown by this experiment that fibrinogen is still present in washed and thrombin treated platelets which carry out release after exposure to calcium ions Thus the action of thrombin, al

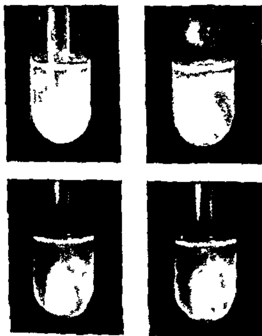


Fig 9 Release and spontaneous coagulation of f[illegible]

[illegible] warmed to 37° C 0 5 ml of 2 × 10 M CaCl was added which produced coarse flocculation in about 15 seconds After 7 minutes at 37° C a solid clot had formed from the released fibrinogen (I) Strong adherence to the glass prevented [illegible] Th s however [illegible]

a moderate release takes place A second release is initiated, which is independent of the enzyme, when calcium is added to the system This release is massive, and is accompanied by an aggregation of the cells which is indistinguishable from viscous metamorphosis Liberation of platelet fibrinogen takes place at this stage

A further observation must now be described *Platelet fibrinogen liberated by treatment with trypsin and subsequent exposure of the cells to calcium ions coagulates spontaneously in about 10 min at 37° C The clot formed carries out retraction*

It is premature to discuss these results until sufficient information has been obtained concerning the mechanism of the release reaction (Chapter 6) In passing it may be pointed out that the attachment of fibrinogen to platelet structures must be of a special character, since this protein is liberated although there is no lysis and other proteins are almost completely retained by the cells Another point of interest is that sufficient prothrombin and its activators are present in washed and trypsin treated cells to enable spontaneous coagulation of the liberated fibrinogen

A question of primary importance, however, is whether these observations can be duplicated with thrombin, since this would represent evidence that platelet fibrinogen does not function as substrate in thrombin catalyzed release Experiments were accordingly performed in which it was attempted to initiate release by calcium ions in platelets pretreated with thrombin

It was found necessary to carry out this incubation of platelets with thrombin at a relatively low temperature (15° C) A calcium complexing agent, citrate or EDTA, must be present in the system Under these conditions thrombin causes no visible alteration of the platelet suspension Evidently, viscous metamorphosis is prevented After incubation thrombin was removed by washing and the suspension was warmed to 37 C Addition of calcium to cells prepared in this manner produced coarse aggregation (viscous metamorphosis) in about 15 seconds A photograph of this phenomenon is shown in Fig 22, Chapter 7 As will be described later (Fig 16, Chapter 6) release takes place simultaneously

During this release, initiated by calcium in platelets previously incubated with thrombin, platelet fibrinogen is liberated In com

addition of calcium elicits a release reaction in which platelet fibrinogen is also liberated Fibrinogen in platelets is therefore resistant towards thrombin as well as trypsin

The complete parallelism between the effects of thrombin and trypsin indicates that these enzymes initiate the release reaction in platelets by acting on a common specific substrate in the cells This substrate is not fibrinogen, which is unaffected by the enzymes No further information is available, however, concerning its identity

though it effectively prepares the platelets for the subsequent *release reaction, does not involve their fibrinogen component*

The close correspondence between the actions of thrombin and trypsin indicates that both *these enzymes initiate the release* reaction in platelets by the same mechanism, namely by hydro lyzing a specific substrate in the cells Neither of these enzymes affects platelet-fibrinogen It is concluded that the release reaction is initiated by their proteolytic effect on a different and as yet unknown protein

SUMMARY OF CHAPTER 4

Further studies are described concerning the state of fibrinogen in blood platelets and the question of its possible role as thrombin *substrate in the release reaction The experiments were carried out* primarily with trypsin, which enzyme has been previously con sidered to have an effect on platelets that is limited to proteins adsorbed upon the surface of the cells

The results obtained with trypsin, however, are not in accord with this view An effect also on structural elements was demon strated by the definite though moderate release of adenine nucleo tides and 5 hydroxytryptamine observed during incubation In addition, treatment with trypsin was found to sensitize the cells *towards calcium ions After removal of the enzyme by washing,* addition of calcium results in a massive release of intracellular constituents (adenine nucleotides and 5 hydroxytryptamine) accompanied by coarse and irreversible aggregation of the cells

Under the conditions of this release reaction platelet fibrinogen is specifically liberated, demonstrating that the preceding trypsin treatment has been without effect on this protein It was addition ally observed that the liberated fibrinogen undergoes a slow, spon taneous coagulation and that the clot thus formed undergoes retraction *It was concluded from these results that fibrinogen is* a true platelet constituent, which is protected from the proteolytic enzyme by its intracellular localization

Subsequent experiments established that identical effects may be obtained with thrombin At low temperature this enzyme has no visible action on platelets provided that calcium ions are trapped with EDTA After removal of the enzyme by washing

in connection with the work on platelet fibrinogen (Chapter 3). This protein was found to be precipitated as a gel from a 0.6 M KCl extract of washed platelets when the ionic strength of the solution was lowered by dilution. The material could be redissolved in 0.6 M KCl, forming a viscid and turbid solution whose most noticeable property was a marked decrease in viscosity upon addition of small amounts of ATP. These properties were recognized as typical of actomyosin and related contractile proteins. Further tests established that this platelet component has the essential characteristics of actomyosin.

In some details the procedure developed in the present work for the preparation of contractile protein from blood platelets differs from that described by BETTEX-GALLAND & LÜSCHER (1961). Thus citrate instead of EDTA is used as anticoagulant, and n-butanol is added during extraction in order to lyse the cells. Furthermore, removal of cell debris by centrifugation of the first extract is carried out at room temperature. If performed in the cold, large losses of contractile protein may occur at this stage. Finally, precipitation of contractile protein is carried out at a relatively high ionic strength (about I = 0.2 μ) in the presence of Mg ions. It is believed that the yield of contractile protein is nearly quantitative under these conditions, although this may be difficult to prove. After two reprecipitations no platelet fibrinogen is present in the preparations, as shown by their failure to clot or flocculate upon addition of thrombin. The ease with which platelets are separated from pig blood, and the efficiency of the extraction, allow small volumes (2 to 4 litres) of citrated blood to be employed for a single preparation.

A description is given below of the preparation of contractile protein from pig blood platelets, and of the total amounts found in the cells by this method of extraction and fractionation. The contractile protein forms about 15 % of the total protein, or 1–2 % of the wet weight of the cells in human platelets (BETTEX-GALLAND & LÜSCHER 1961). As will be shown, these results correspond closely to the values obtained for the contractile protein in pig platelets.

CHAPTER 5

The Contractile Protein of the Platelets

Adenine nucleotides are characteristic components of blood platelets, occurring in the cells in significant concentrations. Among the nucleotides adenosine triphosphate represents the major fraction, as shown previously in the present work in accord with earlier observations by other investigators (BESTETTI & CROSTI 1955, BORN 1956 a, MIZUNO, SAUTER & SCHULTZE 1960). The concentration of adenosine triphosphate in washed pig platelets, determined by ion-exchange chromatography, was found to be 2.1 to 3.1 $\times$ 10^{-3}M in the present study (Chapter 2). These values approach those reported from striated muscle.

At the time of its discovery no function had been established for the high concentration of adenosine triphosphate (ATP) in the platelets. A valuable suggestion was given by BESTETTI & CROSTI (1955). With reference to a platelet protein fraction described by LUSCHER (1953), which had been assumed to be responsible for the process of clot retraction, these investigators proposed that *ATP is functionally connected with a contractile protein in the cells*, in analogy with the role of ATP in muscle.

The correctness of this assumption has been established by the recent demonstration by BETTEX-GALLAND & LUSCHER (1959, 1961) of the presence of an actomyosin like contractile protein in human platelets. The platelet material was found to be closely similar to actomyosin and related contractile proteins in physical properties. Its ATP-ase activity was remarkably low, in which respect the contractile platelet protein resembles the contractile protein of undifferentiated cells described by HOFFMANN BERLING (1956).

In the course of the present studies a primitive contractile protein, whose properties correspond to those of the contractile protein in human platelets (BETTEX GALLAND & LÜSCHER 1959, 1961), was independently isolated and identified in pig platelets

in connection with the work on platelet fibrinogen (Chapter 3) This protein was found to be precipitated as a gel from a 0 6 M KCl extract of washed platelets when the ionic strength of the solution was lowered by dilution The material could be redissolved in 0 6 M KCl, forming a viscid and turbid solution whose most noticeable property was a marked decrease in viscosity upon addition of small amounts of ATP These properties were recognized as typical of actomyosin and related contractile proteins Further tests established that this platelet component has the essential characteristics of actomyosin

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PREPARATION OF CONTRACTILE PROTEIN FROM PLATELETS

In principle the preparation of contractile protein follows closely the method previously described for the preparation of platelet-fibrinogen (Chapter 3). The only difference made in the procedure was to lower the ionic strength in the first precipitation to I = 0.2 μ, in order to ensure the complete precipitation of contractile protein.

1. EXTRACTION. *Platelets obtained from citrated pig blood are* washed 3 times in citrated saline in the cold. The cells sedimented from the last washing are weighed and 2 ml of cold 0.6 M KCl, which is 0.015 M with respect to Tris-buffer of pH 7.5, are added per g of wet cells. After suspension of the cells 25 μl of n-butanol are added *per ml of final solution. Lysis of the cells occurs when* these ingredients are mixed. There is a transient flocculation of platelet proteins, then a smooth solution is formed. Extraction is allowed to proceed for 24 hours in the cold (4° C).

2. PRECIPITATION. The extract is warmed to room temperature and centrifuged at 5000 × *g* for 4 minutes without cooling. Small amounts of insoluble material are removed. The supernatant solution is cooled again, 2 volumes of cold 0.002 M $MgSO_4$ are added and pH is adjusted to 6.5 with 1 % acetic acid. The mixture is kept at 4° C for 1 hour and is then centrifuged at 7000 × *g* for 5 minutes. The contractile protein is sedimented as a stiff gel. This is dissolved in about 2 volumes of 0.6 M KCl solution.

3. REPRECIPITATION. Small amounts of insoluble material are *removed from the solution by centrifugation at 7000 × g for 5* minutes. The contractile protein is reprecipitated by dilution with 2 volumes of cold 0.002 M Mg. Solution in 0.6 M KCl and repre-

Table IV

THE CONTENT OF CONTRACTILE PROTEIN IN PLATELETS

		Contractile protein mg/g platelets
Prep. 1	twice pptd	15.5 mg
» 2	» »	16.7 »
» 3	three times pptd	16.0 »

Contractile protein from three preparations of washed platelets (wet weights 3.0, 3.1, and 5.8 g) was isolated as described in the text. Aliquots of 0.5 ml were employed for protein determination.

cipitation are repeated once Fibrinogen initially present in the preparation is hereby removed Finally the material is dissolved in 0 6 M KCl to the volume of the original platelet extract It can be stored for a few days in the refrigerator

As shown in Table IV the yield of contractile protein obtained by this procedure is about 36 mg per g of wet cells, which represents approximately 20 % of their total protein

PROPERTIES OF THE CONTRACTILE PROTEIN

Actomyosin from striated muscle, the related protein in smooth muscle such as uterus (NEEDHAM & CAWKWELL 1956, and others) and the primitive contractile protein demonstrated in undifferentiated cells by HOFFMANN-BERLING (1956) are characterized by their ability to interact specifically with ATP In the presence of this substance the solubility of the proteins is increased, the viscosity of their solutions is strikingly lowered, and precipitates of the proteins undergo contraction (superprecipitation) These alterations in the proteins are accompanied by an enzymatic effect on the nucleotide, which results in the splitting of its terminal phosphate bond and the release of inorganic phosphate As will

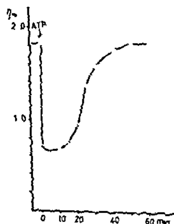


Fig 10 Alterations in specific viscosity of the contractile protein produced by ATP The viscosity of a solution of freshly prepared contractile protein in 0 6 M KCl buffered to pH 7 5 with 0 015 M Tris was determined at 22° C before and after addition of ATP to a final concentration of 2 5 $\times 10^{-3}$M The concentration of contractile protein was 4 mg per ml

be shown below, the platelet protein interacts with ATP in an identical manner, which demonstrates its nature as a contractile protein

The effect of ATP on the viscosity of solutions of the contractile platelet protein is shown in Figure 10

Addition of ATP causes an immediate fall in the viscosity, which gradually returns to its original high value In the experiment above the viscosity number $Z\eta$ is 0 26 and the ATP-sensitivity 107 % calculated after PORTZEHL, SCHRAMM & WEBER (1950) With regard to viscosity and the degree to which this is decreased by ATP the platelet protein is therefore comparable to actomyosin from striated muscle

Actomyosin is insoluble in salt solutions below the ionic strength I = 0 3 μ In the presence of ATP its solubility is increased, the limit of solubility being lowered to approximately I = 0 1 μ The same phenomenon was observed in studies of the contractile platelet protein, as shown in Table V Apparently the limiting ionic strengths for this protein in the presence and absence of ATP are similar to those of actomyosin

Table V

EFFECT OF ATP ON THE SOLUBILITY OF CONTRACTILE PROTEIN *IN DILUTE SALT SOLUTIONS*

	Ionic strength				
	0 30 μ	0 20 μ	0 12 μ	0 08 μ	0 04 μ
Without ATP	—	×	×	×	×
With ATP 5 × 10 ⁴ M	—	—	—	×	×

A series of tubes was arranged containing equal volumes of 0 3 to 0 04 M KCl 1 × 10 ³M Mg and Tris pH 7 0 1 × 10 ³M A second series contained in addition 5 × 10 ⁴M ATP The concentration of contractile protein in the tubes was 0 7 mg per ml Tp 22° C Precipitation (×) in the different tubes was registered after 15 min

In the experiment of which the results were given in Table V, and generally when solutions of the contractile protein were diluted to ionic strengths of 0 1 μ or less, ATP markedly affected the appearance of the precipitated protein Without ATP large and loose floccules were formed, while in its presence the particles were small, granular, and compact This was taken as evidence of superprecipitation in the presence of ATP, an essential property of actomyosin and related contractile proteins

As shown in Figure 11 (upper curve) the platelet protein has a definite but low activity as an ATP-ase

Calculated from this experiment the ATP ase activity of the preparation was 0 012 μM P/mg N/min at 22° C, which is a very low value It corresponds quite closely, however, to the activities found by HOFFMANN-BERLING (1956) for preparations of contractile protein from undifferentiated cells (0 009 to 0 024 μM P/mg N/min) under identical conditions. Similar activities were found by BETTEX GALLAND & LÜSCHER (1961) for contractile protein from human platelets Because of this low enzymatic activity of the contractile platelet protein it is necessary to exclude the possibility that the effect is due to contamination with enzymes of different nature Unspecific ATP-ases are little affected by the actomyosin inhibitor Mersalyl The preparation of contractile protein from platelets, however, is almost completely inhibited, as shown in Figure 11 (lower curve)

It was pointed out by HOFFMANN-BERLING (1956) that a correlation exists between the observed velocity of contraction in different contractile tissues and the enzymatic activity of the respective contractile proteins According to this principle the contractions to be expected in blood platelets are slow, comparable in velocity to the primitive movements of undifferentiated cells, since the contractile proteins of these cells and of platelets are about equally active as ATP-ases On the other hand, the force

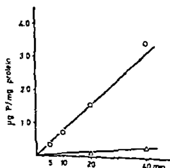


Fig 11 Contractile protein ATP ase act vity and its inhibition with Mersalyl 0 77 mg of contractile protein in a total volume of 1 0 ml was incubated with 5×10^{-3} M ATP at pH 7 0 and 22° C as described under Material and methods Lower curve (△) shows the results obtained in the presence of $2\ 5 \times 10^{-4}$ M Mersalyl

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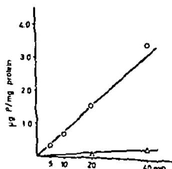


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of contraction is probably much higher in platelets, since these cells contain relatively large amounts of contractile protein Indeed the content of contractile protein in platelets (16 mg per g wet weight) approaches that found in smooth muscle, a fact previously stressed by BETTEX-GALLAND & LUSCHER (1961)

Apart from their participation in the process of clot retraction, whose characteristics differ considerably from muscular contraction, the platelets have no known mechanical functions It is therefore truly remarkable to find that they are constituted like strongly contractile cells The observation clearly suggests that contraction must be taken into consideration as a possible component in platelet reactions On the other hand, it remains to be established how contraction may be initiated and what effects it may produce in these cells

SUMMARY OF CHAPTER 5

The preparation from pig blood platelets of a contractile protein is described The properties of this protein correspond to those of the contractile protein previously isolated by other investigators from human blood platelets

The contractile protein is extracted with 0 6 M KCl buffered to pH 7 5, with addition of n butanol in order to lyse the cells, Precipitations are carried out at I = 0 2 μ, pH 6 5 and tp 4° C, in the presence of Mg ions The yield of purified protein free of platelet fibrinogen is about 16 mg per g of wet cells, or about 20 % of their total protein content

The viscosity of this protein is decreased and its solubility is increased by low concentrations of ATP Superprecipitation was observed below I = 0 1 μ in the presence of ATP The protein has a definite, but low ATP-ase activity which is almost completely inhibited by low concentrations of Mersalyl

The presence of these properties demonstrates that the isolated platelet protein is contractile and that it closely resembles the primitive contractile protein responsible for movements in undifferentiated cells The concentration of contractile protein, however, is about 10 times higher in platelets than it is in undifferentiated cells, indicating that platelets may be able to perform strong contractions

CHAPTER 6

The Different Steps of the Release Reaction

The properties of the release reaction described so far in the present work do not allow a definition of its mechanism. However, this specific cellular reaction is initiated by an effect of thrombin on a substrate in the platelets. The question may therefore be raised whether the process can be satisfactorily explained from the characteristics of a simple proteolytic reaction, or whether additional mechanisms are involved.

Observations have already been made (Chapter 4) which suggest that release is not a direct result of the proteolytic process. Thus it was demonstrated that platelets treated with trypsin and subsequently exposed to calcium ions carry out a release reaction which is indistinguishable from that which can be initiated with thrombin. The experiments also indicated that the thrombin catalyzed process can be similarly separated into two different steps. From these results it may be suspected that the proteolytic step of the release process is followed by a separate reaction which requires calcium, and that this secondary reaction causes liberation of intracellular material. If confirmed, this means that release takes place by a reaction sequence in which the thrombin catalyzed step is the first link.

It was realized that it might be possible to differentiate between a single stage and a multi stage process by following the time course of the reaction. Experiments based on this principle were performed in order to confirm whether a sequential reaction system is in operation during thrombin catalyzed release.

EVIDENCE OF SEQUENTIAL REACTIONS IN THE RELEASE PROCESS

The reaction velocity of a single stage process, $R_1 + R_2 \longrightarrow$ Products is evidently maximal at the beginning of the reaction, because the concentration of reactants is then highest. This con

of contraction is probably much higher in platelets, since these cells contain relatively large amounts of contractile protein Indeed the content of contractile protein in platelets (16 mg per g wet weight) approaches that found in smooth muscle, a fact previously stressed by BETTEX-GALLAND & LUSCHER (1961)

Apart from their participation in the process of clot retraction, whose characteristics differ considerably from muscular contraction, the platelets have no known mechanical functions It is therefore truly remarkable to find that they are constituted like strongly contractile cells The observation clearly suggests that contraction must be taken into consideration as a possible component in platelet reactions On the other hand, it remains to be established how contraction may be initiated and what effects it may produce in these cells

SUMMARY OF CHAPTER 5

The preparation from pig blood platelets of a contractile protein is described The properties of this protein correspond to those of the contractile protein previously isolated by other investigators from human blood platelets

The contractile protein is extracted with 0 6 M KCl buffered to pH 7 5, with addition of n-butanol in order to lyse the cells, Precipitations are carried out at I = 0 2 μ, pH 6 5 and tp 4° C, in the presence of Mg ions The yield of purified protein free of platelet fibrinogen is about 16 mg per g of wet cells, or about 20 % of their total protein content

The viscosity of this protein is decreased and its solubility is increased by low concentrations of ATP Superprecipitation was observed below I = 0 1 μ in the presence of ATP The protein has a definite, but low ATP-ase activity which is almost completely inhibited by low concentrations of Mersalyl

The presence of these properties demonstrates that the isolated platelet protein is contractile and that it closely resembles the primitive contractile protein responsible for movements in undifferentiated cells The concentration of contractile protein, however, is about 10 times higher in platelets than it is in undifferentiated cells, indicating that platelets may be able to perform strong contractions

The course of the thrombin catalyzed reaction, indicated by the broken curve, is assumed to proceed linearly until it is nearing completion The solid curve describes release, resulting from one or more subsequent reactions which are dependent on the preceding proteolytic step The duration of the initial phase of the release reaction (0 to x on the abscissa) will be determined by the velocity of the thrombin-catalyzed step

Examples of the time course of the release reaction have been shown previously (Figs 1 and 5, Chapter 2) From our present point of view these experiments are disappointing, since release appears to proceed linearly from the start. This is the result to be expected, however, if there is a great difference in the velocities of the partial reactions For example, if the thrombin catalyzed step is the most rapid component in the system, characteristic changes in the initial rate of release may be demonstrable only at low concentrations of the enzyme

Experiments with low concentrations of thrombin failed to alter

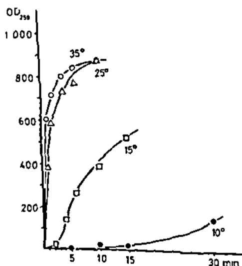
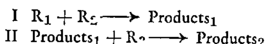


Fig 13 Effect of temperature on the release reaction Samples of platelets (0 05 g) were incubated at 35°, 25°, 15° and 10° C with 0 4 mg of thrombin in 1 ml of Tris buffered saline pH 7 5 which contained 5×10^{-3} M Ca Centrifuxation of the samples was carried out at times indicated on the abscissa Released adenine nucleotides were determined in 0 5 ml aliquots of the platelet free supernatant solutions.

trasts with the progress of a multi-step reaction, in which products formed in the course of the process subsequently play the role of reactants



I $R_1 + R_2 \longrightarrow$ Products$_1$

II Products$_1$ $+ R_3 \longrightarrow$ Products$_2$

Therefore, the velocity of all reactions except the initial step in a reaction sequence is zero at the start and gradually builds up to a maximal value A multi-step reaction may be recognized by this characteristic

When this is applied to the question of the nature of the release reaction it may be stated that release, if it is simply determined by the reaction of thrombin with its substrate, should have its highest velocity immediately after addition of the enzyme to the suspension On the other hand, if release is effected by a sequence of processes, its initial velocity must be zero, and maximal velocity is reached gradually It will be recognized, however, that difficulties may exist, which prevent the demonstration of this difference between single-stage and multi stage reactions Thus, if the individual reactions in a sequence have markedly different velocities, the stage of gradually increasing rate of reaction may virtually disappear

A theoretical example, based on the assumption that a reaction between thrombin and its substrate initiates the process, and that a subsequent reaction directly effects release, is shown below (Fig 12)

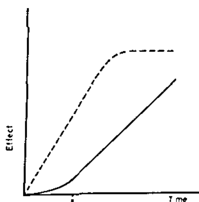


Fig 12 Hypothetical curves the reaction of thrombin with its substrate and the subsequent release Broken line proteolytic reaction catalyzed by thrombin Solid line release (See text)

The course of the thrombin catalyzed reaction, indicated by the broken curve, is assumed to proceed linearly until it is nearing completion The solid curve describes release, resulting from one or more subsequent reactions which are dependent on the preceding proteolytic step The duration of the initial phase of the release reaction (0 to x on the abscissa) will be determined by the velo city of the thrombin-catalyzed step

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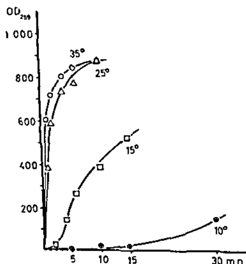


Fig 13 Effect of temperature on the release reaction Samples of [illegible]

[illegible] in 0 5 ml aliquots of the platelet free supernatant solutions

the initially linear time course of the release reaction The effect of different temperatures was therefore tested, and the results shown in Figure 13 were obtained

These curves show that release occurring between 35° and 25° C apparently proceeds with maximal velocity from the start of the reaction Decreasing the temperature from 25° to 15° C causes a profound change in the time-course of release An initial phase of accelerating rate of release then appears, which becomes very pronounced when the temperature is decreased to 10° C

As previously pointed out, an initial phase of rising reaction rate *indicates the operation of a multi-stage process, whose final* product, in the present instance release, is being measured The marked influence of temperature can be explained by the occurrence of a series of linked reactions of different temperature coefficients Both phenomena are in accord with the theory of a sequential reaction system responsible for release

In this reaction sequence, of which there is still only indirect evidence, the reaction between thrombin and its substrate in the platelets is known It can be demonstrated that this enzymatic reaction represents the first stage in the sequence, and that throm-

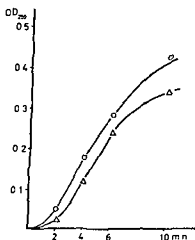


Fig 14 The duration of the lag phase of the release reaction at different thrombin concentrations *Samples of platelets (0 05 λ) were incubated at 15° C* with 0 12 mg and 0 36 mg of thrombin in 1 ml of Tris buffered saline pH 7 5 which contained 5×10^{-3} M Ca Centrifugation of the samples was carried out at times indicated on the abscissa Released adenine nucleotides were determined in 0 5 ml aliquots of the *platelet free supernatant solutions* Samples with 0 12 mg of thrombin △ 0 36 mg of thrombin ○

bin does not directly catalyze the liberation of intracellular material These conclusions were drawn from the experiment shown in Figure 14

In this experiment the course of release was followed at two concentrations of thrombin It is seen that the duration of the initial phase is reduced at the higher concentration of enzyme A higher velocity of the thrombin catalyzed step thus shortens the delay before maximal rate of release is obtained, although the final velocity reached is the same at both concentrations of thrombin

It can be concluded that thrombin initiates the release reaction, and that the thrombin catalyzed step is not rate limiting at the concentrations of enzyme employed Furthermore, thrombin does not participate in the final mechanism of liberation of intracellular material, which is therefore effected by a separate process

The validity of these conclusions is not influenced by the nature of the secondary step or steps which follow upon the reaction of thrombin with its platelet substrate For example, all characteristics so far observed may be explained from the participation of diffusion in the release process On the other hand, in order to determine whether diffusion or processes of entirely different character serve as links in the release reaction, its individual steps must be separated from each other

THE DEPENDENCE OF RELEASE ON CALCIUM IONS

Observations suggesting a dependence of the release reaction on calcium have already been described (Chapter 4) in connection with studies of the action of trypsin and thrombin on platelet-fibrinogen The explanation of this effect is given by the studies described below in which a separation of the proteolytic step from the subsequent reactions of the release process was obtained in incubations with thrombin in the absence of calcium ions

For reasons discussed later calcium complexing agents have little effect on release when the incubation is carried out at high temperatures At 15° C, however, citrate or EDTA will completely prevent the occurrence of release (Fig 15)

This is not due to an inhibition of the reaction of thrombin with its substrate in the platelets but to a block introduced into the reaction sequence after the thrombin catalyzed step, as shown in Figure 16 It is seen from the latter experiment in which incuba

tion with thrombin was carried out in the presence of EDTA, that no release takes place as long as calcium ions are absent When a surplus of calcium is added, however, release starts immediately Significantly, velocity of release is now maximal from the beginning Evidently, preincubation with thrombin in the absence of calcium ions has allowed the thrombin-catalyzed step to reach a stage which is sufficient for maximal release velocity

This shows that the enzymatic attack of thrombin on platelets is independent of calcium ions A subsequent step, which is the process responsible for release, is completely dependent on calcium The release reaction is therefore a sequential process, whose first step is catalyzed by thrombin (and trypsin) and whose following step requires calcium ions

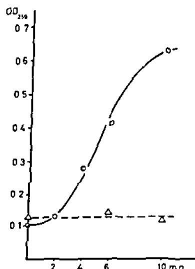


Fig 15 Inhibition of release with citrate at 15° C Samples of platelets (0 05 g) were incubated at 15° C with 0 4 mg of thrombin in 1 ml of Tris buffered saline pH 7 5 Two series were employed one containing 5 × 10⁻³M Ca and the other 3 × 10⁻³ M sodium citrate Centrifugation was carried out at times indicated on the abscissa Released adenine nucleotides were determined in 0 5 ml aliquots of the platelet free supernatant solutions Solid line incubation with calcium ions Broken line incubation with citrate

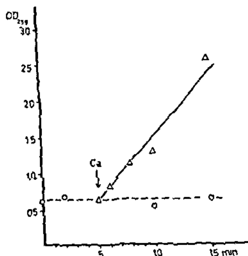


Fig 16 Release initiated with calcium ions in platelets preincubated with thrombin in the presence of EDTA Samples of platelets (0 05 g) were incubated at 15° C with 0 4 mg of thrombin in 1 ml of Tris buffered saline pH 7 5 containing EDTA 1 25 × 10 ³M Two series were employed, one of which was recalcified after 5 min of incubation with thrombin to a final concentration of 3 75 × 10 ³M Ca Centrifugation of the samples was carried out at times indicated on the abscissa Released adenine nucleotides were determined in 0 5 ml aliquots of the platelet free supernatant solutions Samples incubated without recalcification are marked ○, recalcified samples △

THE PARTICIPATION OF CONTRACTION IN THE RELEASE REACTION

The nature of the secondary step or steps which follow upon the thrombin catalyzed reaction in the release process cannot be deduced from presently available information A direct chemical reaction between some structural element of the platelets and calcium can be imagined A second possibility is an enzymatic reaction which requires calcium ions

It is, however, undoubtedly necessary in all studies of the functional properties of platelets to take into consideration that they contain both ATP and a contractile protein, and that they are therefore potentially contractile cells. In the previously mentioned studies of BETTEX GALLAND & LÜSCHER (1960, 1961), the contractile protein of platelets was suggested to be involved in viscous metamorphosis and clot retraction Experiments which they carried out in order to confirm this theory, however, were equivocal

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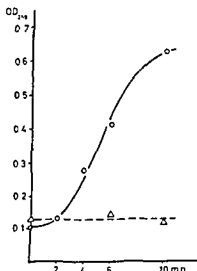


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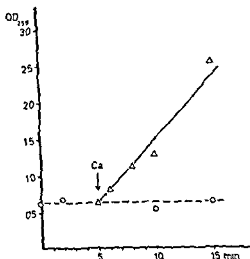


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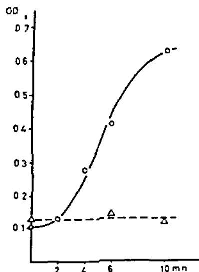


Fig. 15. Inhibition of release with citrate at 15° C. Samples of platelets (0.05 g) were incubated at 15° C with 0.4 mg of thrombin in 1 ml of Tris buffered saline pH 7.5. Two series were employed: one containing 5×10^{-3} M Ca and the other 3×10^{-2} M sodium citrate. Centrifugation was carried out at times indicated on the abscissa. Released adenine nucleotides were determined *in 0.5 ml* aliquots of the platelet free supernatant solutions. Solid line: incubation with calcium ions. Broken line: incubation with citrate.

The experiment demonstrates a small reduction of ATP in the cells incubated with thrombin at 37° C, amounting to 20 % of the initial concentration in the cells At 15° C, however, at which temperature release certainly takes place, an apparent increase in ATP is seen

A decrease in platelet ATP is not in itself sufficient evidence for the occurrence of contraction, since it may result from the action of unspecific ATP-ases independently of the enzymatic activity of the contractile protein In the present experiment (Fig 17) the fall in the ATP-concentration at 37° C is limited, and its time-course differs from the course of the release reaction At 15° C there is an increase rather than a loss in platelet ATP, although the cells do contract at this temperature if contraction is an element in the release reaction Evidently, these results do not reveal the occurrence of contraction in platelets exposed to thrombin On the other hand, it is not in any way excluded that contractions take place, since a loss of ATP may be completely masked by simultaneous resynthesis Synthesis of ATP concurrently with its breakdown was in fact observed by BETTEX GALLAND & LUSCHER (1960) in human platelets after addition of thrombin In this situation the alterations in ATP concentration evidently

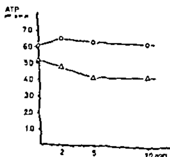


Fig 17 The ATP concentration of platelets in release Samples of platelets 0 10 g) were incubated at 15° and 37° C with 0 3 mg of thrombin in 1 ml f Tris buffered saline pH 7 5 which contained 2 5 × 10 ³M Ca The reaction was terminated at the times indicated on the abscissa by addition of 1 0 ml of cold 5 % perchloric acid The tubes were left in ice water for 5 min … centrifuged Aliquots of the de[illegible]
of a predetermined v[illegible]
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These negative results, on the other hand, do not exclude the possibility that contractions take place in platelets There is, in the sudden crumbling of platelet structures apparent in the release phenomenon, a suggestion of force which contraction presumably would be able to deliver

There are not many methods available for the registration of contraction in cells It can only be demonstrated with certainty by direct observation of shortening, which is probably impossible in platelets Certain alterations in morphology, appearing in platelets exposed to thrombin, may indirectly indicate the occurrence of contraction As proof of platelet contraction these alterations must be regarded as useless There remains only the possibility of demonstrating the eventual occurrence of contraction in platelets biochemically

It is generally accepted that muscular contraction involves an interaction between actomyosin and ATP, of such a nature that energy liberated by hydrolysis of the terminal phosphate bond of the nucleotide is transformed into mechanical work A decrease in ATP, with parallel formation of ADP plus inorganic phosphate should therefore accompany contraction Unfortunately, living cells appear to resynthesize ATP with a velocity equal to its breakdown Alterations in the concentrations of phosphate and nucleotides have not been found demonstrable in cells under normal conditions

It was claimed by BORN (1956 b), however, that ATP is broken down in platelets in connection with clotting BETTEX GALLAND & LUSCHER (1960) observed a fall in ATP concentration to about 50 % of the initial value during 30 min of incubation of fresh platelets with thrombin The results suggest that in platelets in contrast to other living cells hydrolysis of ATP during contraction may be demonstrable Its possible occurrence during the release reaction was therefore investigated

In this experiment (Fig 17) platelets were incubated with thrombin at 15° and 37° C in the presence of calcium ions for periods of up to 10 min The reaction was stopped by deproteinization with cold perchloric acid and the deproteinized extract was neutralized after 10 min ATP was determined enzymatically by the coupled oxidation of DPNH in a phosphoglycerate kinase / glyceraldehydephosphate dehydrogenase reaction system

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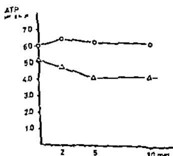


Fig 17 The ATP concentration of platelets in release Samples of platelets (0 10 g) were incubated at 15° and 37° C with 0 5 mg of thrombin in 1 ml of Tris buffered saline pH 7 5 which contained 2 5 × 10 3M Ca The reaction was terminated at the times indicated on the abscissa by addition of 1 0 ml of cold 5 % perchloric acid The tubes were left in ice water for 5 min and centrifuged Aliquots of the deproteinized solutions were neutralized by addition of a predetermined volume of cold 1 25 M K_2CO_3. Precipitated potassium per chlorate was removed by decantation and the samples were frozen until analyzed for ATP with the phosphoglycerate kinase/glyceraldehydephosphate dehydro genase system Samples incubated at 37° are marked △, at 15° ○

do not express the kinetics of contraction The latter authors observed a somewhat greater reduction of ATP (about 50 % of the initial concentration) in a system characterized by very high platelet concentrations, rather low thrombin and addition of magnesium *instead of calcium* These conditions do not appear more favourable to the reaction than those employed in the present studies The lack of agreement therefore probably reflects species differences between human and pig blood platelets, particularly with regard to the metabolic activity responsible for the synthesis of ATP

It must be concluded that it is not possible to demonstrate contraction in platelets by following alterations in their ATP content Evidence that contraction is a step in the release reaction was obtained, however, by observations on the effect of inhibitors of actomyosin For reasons to be discussed actomyosin poisons have *only a weak effect on platelets at 37° C At 15° C the inhibitors* Mersalyl and p chloromercuribenzoate (KIELLEY & BRADLEY 1956) were found to be strongly inhibitory towards the release reaction as shown in Figure 18

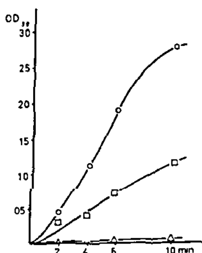


Fig 18 Effect on release of inhibitors of contraction (Mersalyl and PCMB) Samples of platelets (0 05 g) were incubated with 0 4 mg of thrombin in 1 25 ml of Tris buffered saline pH 7 5 at 15° C in the presence of 4×10^{-3}M Ca The curves show the course of release in this system ○ and in systems additionally containing 5×10^{-4}M Mersalyl □ or 5×10^{-4}M PCMB △ The samples were centrifuged at times shown on the abscissa Released adenine nucleotides were determined in 0 5 ml of the platelet free supernatant solutions

A difference is evident in the molar activities of Mersalyl and PCMB, the latter substance producing practically complete inhibition of the release reaction at 5×10^{-4} M Probably this difference is determined by the velocities with which these substances are able to penetrate into the platelets

Inspection of the curve for the release reaction partially inhibited with Mersalyl, in Figure 18, shows the duration of the initial phase, until maximal rate of release is obtained, to be unaltered Mersalyl therefore acts upon the final step in the sequence of reactions which is directly responsible for the release phenomenon

This action of Mersalyl, and of PCMB, was accepted in the present work as proof that contraction of the platelets, initiated by a preceding effect of thrombin on the cells, is the last stage in the release process

THE FUNCTIONAL CONNECTIONS BETWEEN PROTEOLYSIS, CA EFFECT AND CONTRACTION

As shown by the experiments described above, the partial processes of the release reaction can be defined in the following terms

Step 1 Thrombin catalyzed proteolysis of the platelet substrate
» 2 The calcium requiring reaction
» 3 Contraction of the platelet
» 4 Liberation of intracellular *material*

The functional connections between these different steps remain to be established and the first question to be raised concerns the relation between the proteolytic step and calcium Why do calcium ions produce release in platelets which have been exposed to thrombin (or trypsin) although they have no such effect on intact cells?

It has previously been shown that removal of calcium ions (Fig 15) or addition of the actomyosin inhibitor PCMB (Fig 18) is able to prevent the occurrence of release at 15° C It is a specific property of the release reaction that this inhibition is almost completely abolished at higher temperatures This is shown by the following experiment (Table VI), in which the platelets were incubated at 37° C

Table VI

INHIBITION OF RELEASE WITH EDTA AND PCMB AT 37°

	Increase in optical density (OD) during incubation with thrombin		
	2 min	5 min	10 min
1 No additions	0 390	0 393	0 420
2 With 5×10^{-3} M Ca	520	530	560
3 » 5×10^{-3} M EDTA	243	250	280
4 » 5×10^{-4} M PCMB	205	255	315

Platelets (0 05 g) were incubated at 37° C with 0 5 mg of thrombin in 1 5ml of Tris buffered saline pH 7 5 Parallel samples contained 5×10^{-3} M Ca 5×10^{-3} M EDTA or 5×10^{-4} M PCMB and were preincubated with these substances for 10 min at 20° C Incubation was started by addition of thrombin and was terminated after 2 5 and 10 min by centrifugation Released adenine nucleotides were determined in 1 ml aliquots of the platelet free supernatant solutions

In spite of preincubation, substantial release takes place in the presence of EDTA or PCMB at this higher temperature There must therefore be a special explanation of the fact that these substances are completely inhibitory at 15° C

It may be assumed that intact platelets, like cells in general, are impermeable to EDTA and PCMB The fact that inhibition is observed in incubations carried out at 15° C therefore shows that penetration is made possible by a process which is part of the release reaction It must be the first step, the reaction of thrombin with the cells, which causes this increase in platelet permeability, and thereby enables the inhibitors to diffuse into the cells and block subsequent reactions Apparently, at 37° C penetration of EDTA and PCMB into the platelets is slow in relation to the high velocity of the release reaction Only a limited inhibition is therefore produced at this temperature

It may be concluded that *an increase in platelet permeability is produced by thrombin*, after which substances originally excluded by permeability barriers are able to penetrate into the cells Furthermore, from the absolute requirement for calcium ions in the release reaction (Figs 15 and 16) and the demonstrated effect of thrombin on platelet permeability *it may be assumed that one function of the thrombin catalyzed step consists in allowing cal-*

cium to penetrate into the cells The lack of effect of calcium ions on intact platelets is then reasonably explained as an expression of inability to cross the surface barrier

It is implied in this theory of the function of thrombin in the release reaction that free calcium ions are absent in intact platelets, on which point there is no known information It is probable, however, that the concentration of calcium in platelets is at least very low, since 80 % of the amount of this element in the cells has been shown to be lipid bound and non-exchangeable (WALLACH, SURGENOR & STEELE 1958) Also, addition of calcium to the medium in which the release reaction is carried out definitely stimulates the process An example of this effect is shown in Table VII

However, the same experiment demonstrates that release takes place at 37° C without added calcium, even in the presence of EDTA, which must effectively remove traces possibly present as contaminants These results apparently contrast sharply with the obligatory participation of calcium postulated above, and it must be considered how they can be included in the theory of the action of thrombin

An experiment was carried out in order to establish with cer-

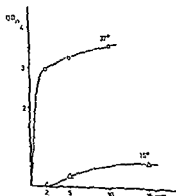


Fig 19 Release in the absence of extracellular calcium Thrombin was dissolved to a concentration of 1 mg per ml in 0 9 % NaCl. The solution was decalcified by passing twice through a 0 5 X 1 cm column of *Dowex 50 – X8* in the sodium form 1 ml samples of platelets (0 05 g), suspended in Tris buffered saline pH 7 5 were incubated with 0 5 *ml* of the decalcified thrombin solution at 15° and 37° C. Centrifugation of the samples was carried out at the times indicated on the abscissa Released adenine *nucleotides* were determined in 1 ml of the platelet free supernatant solutions.

tainty that release can take place in the absence of calcium derived from the medium The solution of thrombin was passed twice through a short column of Dowex-50 in the Na form, to remove possible traces of calcium All other reagents were of analytical purity and were assumed to be calcium-free No *calcium-complexing* agents were added to the system It is seen from Figure 19 that release takes place at 37° C and, more surprisingly, also at 15° C, although calcium was thus excluded from the incubation medium

It has been shown previously (Figs 15 and 16) that no release takes place at 15° C if citrate or EDTA is present The fact that release occurs in the absence of these calcium-binding agents, when the medium does not contain this element, therefore shows that calcium may be derived from the platelets in sufficient quantities to permit a limited release Evidently it is for this reason that the release reaction can take place at 37° C, even in the presence of EDTA or citrate

In addition to the ability of thrombin to increase platelet permeability it is therefore necessary to postulate that *this enzyme, in reacting with the platelets, is able to mobilize a part of their contained calcium so that it may participate in the release reaction*

The mechanism suggested above, in which thrombin is considered to make the platelets permeable to calcium ions and simultaneously to ionize calcium present in the cells, is the most simple explanation which seems possible for the role of this enzyme in the release reaction The possibility is not excluded, however, that the calcium-requiring step in the release reaction is more directly dependent on thrombin Theoretically it is possible that one of the reactants in this step, whose actual nature is still unknown, must be activated by a preceding reaction with thrombin

The next point which demands consideration in the analysis of the release reaction concerns the action of calcium and its relations to the occurrence of contraction in the cells The observations described above show that contraction of platelets, with consequent liberation of intracellular material, is dependent on calcium ions An interesting analogy here exists with the situation in striated muscle, where contraction seems to be similarly dependent on calcium A brief account will therefore be given of the role of calcium in muscular contraction

Among the conditions necessary for the contractions of muscle models and isolated actomyosin systems a requirement for magnesium ions has been established (WEBER 1958) Calcium ions, on the other hand, are not essential In contrast, living muscle is believed to depend completely on the presence of calcium for contraction This is due to the existence in muscle of an activity (Relaxing factor), which is able to prevent the interaction of ATP and actomyosin, and thus prevent contraction (MARSH 1952, BENDALL 1953, HASSELBACH & WEBER 1953, MUELLER 1960)

The activity of the relaxing factor in striated muscle is completely abolished by low concentrations of calcium ions Thus, the fact that an unstimulated muscle is at rest may be explained by the activity of the relaxing factor, calcium ions presumably being absent On the other hand, stimulation of muscle into contraction probably involves the appearance of calcium ions intracellularly, with consequent interruption of relaxing factor activity (WEBER 1958)

On this basis it may be proposed that platelets are prevented from performing contractions by the presence in the cells of a similar relaxing factor activity, until specifically stimulated by the intracellular appearance of calcium ions By this hypothesis the roles of thrombin and of calcium in the release reaction are seen to be satisfactorily and simply explained The direct demonstration of relaxing factor activity which is necessary to confirm this hypothesis was considered to lie beyond the scope of the present investigation

We have now traced the development of the release reaction, through its proteolytic step and the reaction with calcium to the occurrence of contraction In terms of present theory of muscular contraction this last step involves an interaction between ATP and the contractile protein of the cells, and this produces shortening In order to predict the direct consequences of this intracellular contraction it is necessary to know the localization and molecular orientation of the contractile protein in the platelets These details in the submicroscopical structure of the cells remain to be established It will be considered later, however, in what way platelet contraction may produce the known morphological alterations of the cells

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SUMMARY OF CHAPTER 6

Studies are described concerning the occurrence of intermediate reactions in the thrombin catalyzed release process in platelets and the nature of its different reaction steps

It is pointed out, from observations made previously in the present work, that *thrombin catalyzed release probably involves a sequential reaction system* This is not demonstrable at higher temperatures (25° to 37° C), at which release follows initially linear curves However, at lower temperatures (15° C) sigmoid release curves are observed, which indicates the operation of a *multi stage process An increase in the concentration of thrombin* produces a shortening of the lag phase of the release process, without influencing the maximal velocity of release This confirms that thrombin catalyzes the first step in the sequence of reactions

Experiments demonstrate that this initial, thrombin catalyzed step in the release process takes place independently of the presence of calcium It is followed by a reaction for which calcium ions are essential

The possibility of demonstrating the occurrence of platelet contraction in the course of the release reaction is discussed It is pointed out that alterations in the ATP concentration of the cells are unlikely to appear, because of the ability of the cells to re-synthesize this substance In the present studies no fall in platelet ATP which could be ascribed to platelet contraction was observed under conditions optimal for the release reaction However, at low temperatures (15° C) the actomyosin inhibitors Mersalyl and p chloromercuribenzoate were found to be strongly inhibitory towards release The curves indicate that this inhibition is not directed towards the initial, thrombin catalyzed step of the reac tion It was concluded that these inhibitors act upon a subsequent step which consists in platelet contraction

Experiments with inhibitors (EDTA and PCMB), to which intact platelets may be assumed to be impenetrable, indicated that thrombin produces an increase in the permeability of the cells Diffusion barriers are thus removed, and substances present in the medium may enter the platelets *This is of special importance in* connection with the effect of calcium, since it suggests that throm-*bin initiates contraction by rendering the platelets permeable to*

this ion According to this view it is the entrance of calcium ions into the cells which directly causes platelet contraction Calcium which is already present in the cells is inactive until the platelets have been exposed to thrombin The enzyme does, however, partially mobilize this source of calcium ions and hereby enables its participation in the reaction

The observations suggest that the action of calcium in the reaction may be similar to its role in the contraction of striated muscle In the latter, contraction depends on calcium ions for the abolishment of relaxing factor activity The action of calcium in release may represent an analogous effect upon a relaxing factor in platelets

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The experiment (Figs 20 and 21) was carried out at 15° C At this temperature the release reaction proceeds relatively slowly and has a characteristic initial lag phase, as described previously Incubation was terminated by centrifugation of the samples at times measured from the addition of thrombin, and adenine nucle otides released from the cells were determined in the supernatant solutions During this centrifugation, which is carried out in an angle head, the platelets are thrown against the wall of the centri fuge tube and must slide or roll along this inclined plane before collecting at the bottom In this traverse the cells are pressed against the tube-wall by the centrifugal force Intact platelets demonstrate no adhesiveness under these conditions, and all cells are found in the tip of the tube after centrifugation In contrast, after exposure to thrombin adhesive platelets are observed to stick to the glass within the contact area in amounts depending on their exposure to the enzyme

The course of release in this experiment is shown in Figure 20 There is a short lag phase followed by almost constant velocity of release

The photographs in Figure 21 show the appearance of the centrifuge tubes after centrifugation and removal of the incubation medium

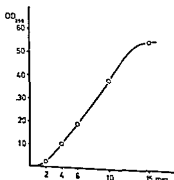


Fig 20 A release exper[illegible] was carried out at the times indicated on the absc ssa Released adenine nucleot des were determined in 0 5 ml of the platelet free supernatant solutions The tubes were subsequently photographed to demonstrate adhering platelets (Fig 21)

CHAPTER 7

The Release Reaction and Viscous Metamorphosis of Platelets

A brief account of the morphological alterations of viscous metamorphosis has been given previously in the present work These changes in platelets are produced by thrombin and are essential in the process of formation of the hemostatic platelet plug Little is known, however, concerning the mechanism responsible for viscous metamorphosis BETTEX GALLAND & LUSCHER (1960, 1961), have suggested that the contractile protein of platelets is involved in the process This view is still unsupported by experimental evidence and has not been worked out in sufficient detail to allow a rational explanation of the causal relationships

In the present investigation the possibility of using the release reaction of the platelets for studies of the mechanism of action of thrombin has been explored It was pointed out in introducing these studies that reasons exist for believing release and viscous metamorphosis to be closely related phenomena Observations which have already been described, notably the simultaneous initiation of viscous metamorphosis and release with calcium ions in platelets previously exposed to thrombin, are clearly in accord with this concept It is therefore probable that these phenomena are different expressions of the same fundamental process in the platelets In view of the obvious importance of being able to explain viscous metamorphosis in terms of the mechanism responsible for the release reaction some experiments were carried out, in which it was attempted to show directly that they are parallel phenomena of identical characteristics In the first of these studies the development of viscous metamorphosis, measured by its most important single property, namely platelet adhesiveness was followed under the conditions employed for registration of release

inhibitory towards release at 15° C (Fig 18), although at 37° C even the most active of these inhibitors has but a small effect (Table VI) These properties were found to be equally characteristic of viscous metamorphosis

The experiment was carried out by incubating platelets with thrombin at 15°, 25° and 35° C, in the presence and absence of Mersalyl Viscous metamorphosis was registered by the appearance of visible platelet aggregates, and the reactions were graded according to the size of the floccules produced The results are shown in Table VII

Table VII

THE EFFECT OF TEMPERATURE AND OF MERSALYL ON THE VELOCITY OF DEVELOPMENT OF VISCOUS METAMORPHOSIS

	3	4	5	6	7	8	9	10	11 min
15°	0	0	Gr	Gr	+	++	+++	Sed	Sed
15° + Mersalyl	0	0	0	0	0	0	Gr	+	++

	1/2	1	1 1/2	2	2 1/2 min
25°	0	++	+++	Sed	Sed
25° + Mersalyl	0	0	0	++	Sed

	10	20	30 seconds
35°	0	+++	Sed
35° + Mersalyl	0	+++	Sed

Samples of platelets (0 05 g) were incubated at 15°, 25° and 35° C with 0 4 mg of thrombin in 1 0 ml of Tris buffered saline pH 7 5 which contained 5 X 10 3 M Ca A second series of samples additionally contained 5 X 10^{-4} M Mersalyl The tubes were rotated slowly by hand and inspected at short intervals against a dark background The Table indicates the degree of platelet aggregation at different time points after addition of thrombin Barely perceptible aggregates are marked (Gr), floccules of increasing size (+, ++, +++), large and sedimenting aggregates (Sed)

Both the length of the latent period before aggregates appear and the time needed for the first, minute floccules to grow into sedimenting aggregates are, as shown in this Table, increased at the lower temperatures Viscous metamorphosis is evidently dependent on temperature in a manner similar to the release reaction Furthermore, Mersalyl is seen to be inhibitory towards viscous metamorphosis at 15° C, whereas it has no effect at 35° C The peculiar temperature dependence of the action of inhibitors is thus found also in viscous metamorphosis

It is clearly seen that adhesiveness is absent in platelets centrifuged before the addition of thrombin (0 min) Adhesiveness becomes increasingly prominent in the course of incubation (2–15 min) The densities of the depositions express the degree of adhesiveness of the platelets at definite time-points after addition of thrombin The result therefore indicates a close parallelism in the developments of viscous metamorphosis and release

A second experiment was performed, in which the effect of different temperatures and of Mersalyl on viscous metamorphosis was studied It will be remembered that a decrease in incubation temperature has an effect on the release reaction characterized by the appearance of an initial lag phase and reduced maximal velocity of release (Fig 13, Chapter 6) Also, Mersalyl and PCMB are

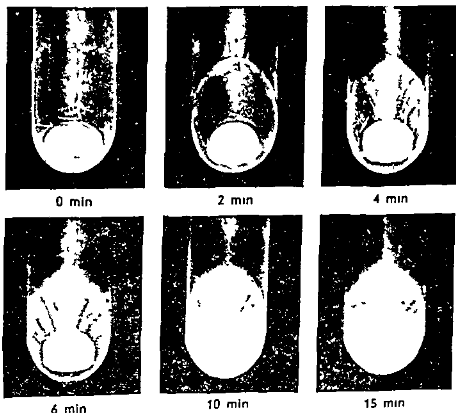


Fig 21 The development of platelet adhesiveness in the course of the release reaction The conditions of the experiment are described under Fig 20 Remaining suspension medium was removed from the centrifuge tubes by decantation In order to demonstrate the layer of platelets adhering to the glass wall the tubes were mounted against a black background under oblique illumination

unaltered Thrombin was removed by washing and the platelets were suspended in 2 5 mM EDTA in saline When this suspension of thrombin treated and washed platelets was warmed to 37° C and an excess of calcium was added, coarse flocculation occurred in 15 seconds Photographs of the thrombin-treated suspension before and after the addition of calcium are shown in Fig 22

This almost immediate formation of platelet aggregates is evidently an expression of viscous metamorphosis, which is taking place in the absence of thrombin It must be concluded that adhesiveness of platelets has no connection with coagulation It is a direct result of the contraction of the platelet, and this is all that is required

The experiments therefore lead to the conclusion that viscous metamorphosis and the release reaction are parallel results of the same fundamental cellular reaction in the platelets, the elements of which have been described in connection with the studies of the release process Both these phenomena are expressions of structural alterations in the cells, produced by contraction of the platelet It is especially to be emphasized that this type of adhesiveness is completely dependent on platelet contraction

SUMMARY OF CHAPTER 7

Investigations were carried out on the connection between the thrombin catalyzed release reaction and viscous metamorphosis of platelets In these experiments viscous metamorphosis was registered by the development of adhesiveness in platelets exposed to thrombin The time course of this process, and its dependence on temperature and on inhibitors of the release reaction were determined

It is shown that adhesiveness, measured by the deposition of adhesive platelets on the lateral wall of centrifuge tubes during centrifugation, develops in close parallel to the release reaction Flocculation of platelet suspensions, also an expression of adhesiveness, is shown to be influenced by temperature and by the inhibitor Mersalyl in the same way as release Finally it is demonstrated that viscous metamorphosis, in the form of irreversible aggregation of platelets, is initiated by calcium ions in platelets

It has been shown previously that the final step in the release reaction consists in contraction of the platelet Thrombin creates the *conditions which allow contraction* and consequent release The present results indicate, perhaps surprisingly, that viscous metamorphosis has exactly the same mechanism The effect of thrombin in viscous metamorphosis is therefore limited to the initial step in the process This is a point of considerable interest, since *the character of viscous metamorphosis, with its marked* aggregation of platelets into cohesive masses, might suggest that the cells are bonded together by a process of coagulation Especially in view of the occurrence of fibrinogen intracellularly in platelets, this hypothesis could have considerable attraction However, *contraction alone appears to be the essential element on which* platelet adhesiveness is dependent

This is brought out especially clearly by the fact that viscous metamorphosis may take place in the absence of thrombin The experiment was carried out by incubation of platelets with thrombin at 15° C in the presence of EDTA As shown previously (Figs 15 and 16, Chapter 6) no release takes place under these conditions It was also pointed out that the suspension remains morphologically



Fig 22 Viscous metamorphosis initiated with calcium in platelets preincubated with thrombin Platelets (0 10 g) were incubated with 1 0 mg of thrombin for 7 min at 15° C in the presence of 1 25 × 10^{3}M EDTA in 2 ml of Tris buffered saline pH 7 5 The cells were collected by centrifugation washed twice in saline with 5 × 10^{1}M EDTA and resuspended in Tris buffered saline pH 7 5 containing 2 5 × 10^{3}M EDTA The smooth suspension was warmed to 37° C and 0 5 ml of 2 × 10^{7}M Ca was added Coarse flocculation of the platelets was observed within 15 seconds after addition of calcium The photo graphs show the thrombin treated and resuspended platelets (a) and the same suspension immediately after the occurrence of *platelet aggregation* (b)

unaltered. Thrombin was removed by washing and the platelets were suspended in 2.5 mM EDTA in saline. When this suspension of thrombin treated and washed platelets was warmed to 37° C and an excess of calcium was added, coarse flocculation occurred in 15 seconds. Photographs of the thrombin treated suspension before and after the addition of calcium are shown in Fig. 22.

This almost immediate formation of platelet aggregates is evidently an expression of viscous metamorphosis, which is taking place in the absence of thrombin. It must be concluded that adhesiveness of platelets has no connection with coagulation. It is a direct result of the contraction of the platelet, and this is all that is required.

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SUMMARY OF CHAPTER 7

Investigations were carried out on the connection between the thrombin-catalyzed release reaction and viscous metamorphosis of platelets. In these experiments viscous metamorphosis was registered by the development of adhesiveness in platelets exposed to thrombin. The time course of this process, and its dependence on temperature and on inhibitors of the release reaction were determined.

It is shown that adhesiveness, measured by the deposition of adhesive platelets on the lateral wall of centrifuge tubes during centrifugation, develops in close parallel to the release reaction. Flocculation of platelet suspensions, also an expression of adhesiveness, is shown to be influenced by temperature and by the inhibitor Mersalyl in the same way as release. Finally it is demonstrated that viscous metamorphosis, in the form of irreversible aggregation of platelets, is initiated by calcium ions in platelets

pretreated with thrombin in the absence of this ion Release takes place simultaneously

It is concluded that the release reaction and viscous metamorphosis are parallel results of the same fundamental process in the platelets The essential elements in this process, a proteolytic step followed by platelet contraction, have been established by the studies on the mechanism of release Clotting of liberated platelet fibrinogen is not essential in viscous metamorphosis, although it evidently takes place when thrombin is present

CHAPTER 8

The Extrusion Theory of Release and Viscous Metamorphosis

A mechanism of contraction has been shown in this work to be responsible for the release phenomenon and for viscous metamorphosis Platelets are thus self destructive cells, in which structural degradation occurs when the cells are stimulated to contraction This unique property of the platelets may represent a universal principle in hemostatic cells An example may be seen in the so-called explosive corpuscles of crustacean blood, which are subject to apparently spontaneous disruption upon extravasation, a process strikingly similar to viscous metamorphosis Indeed, from the violence and speed of the release process in metamorphosing platelets these could be described as the explosive corpuscles of mammals

It must be of importance to the establishment of their specialized structural lability how the contractile protein of platelets is localized within the cell This is not known The mechanical characteristics of the contraction process in the platelets therefore must be identified through the structural effects which are produced

Because of the small dimensions of the platelets, observations with the light microscope can give little information on details of the structural alterations in viscous metamorphosis Only electron microscopical investigations, particularly of sections of platelets, can be expected to reveal the character of these changes

Sections of imbedded, normal platelets were studied by DEMARSH *et al* (1955), PEASE (1955, 1956), RINEHART (1955) and KISCH (1957) As described by these authors the normal platelet is approximately globular, with a smooth outline and a well defined membrane Within the structureless, cytoplasmic matrix various types of cellular organelles are present A number of electron-dense, sharply delineated, large granules are prominent There are

also a few mitochondria and small vesicles. Throughout the cytoplasm, randomly oriented double membranes of irregular outlines and varying lengths are observed.

This picture is fundamentally altered in the course of coagulation, that is in viscous metamorphosis, as shown by the studies of KUHNKE (1958). In coagulation the surface of the platelet becomes highly irregular, due to the *stretching out of numerous processes* of cytoplasm, between which clefts penetrate deeply into the cell. The electron dense granules disappear completely, while other details of the ultrastructure, including the mitochondria, initially remain preserved. A partial disappearance of the surface membrane *was observed. This structure was preserved, however,* in areas of contact between the platelets, indicating that there is no actual fusion of the cells in viscous metamorphosis. According to KUHNKE these alterations convey the impression that the cells were arrested by fixation while performing extremely active movements. This interpretation evidently agrees with the conclusions drawn in the present studies, according to which viscous metamorphosis is produced by platelet contraction.

As already pointed out, KUHNKE (1958) observed a partial loss of the surface membrane in metamorphosed platelets. This contrasts with observations of RODMAN, MASON, McDEWITT & BRINKHOUS (1961), who found this structure to remain preserved. More detailed investigations of this point seem to be indicated. Since, however, an apparent loss of portions of the surface membrane of the platelets could possibly arise during preparation, it will be assumed in the present discussion that this membrane remains attached to the cells during metamorphosis. This evidently does not exclude that the surface membrane of the platelets may undergo *chemical alterations. Such alterations certainly occur* during the exposure of the cells to thrombin although they are not revealed by the electron microscope.

On the basis of these morphological observations the release reaction of the platelets may be defined as a cellular process occurring in contracting cells whose submicroscopical structure is undergoing extensive degradation while the continuity of the surface membrane remains *preserved. It has been shown previously in the* present work (Chapter 6) that the permeability of this surface membrane of platelets is increased during the reaction of the cells

with thrombin This factor alone does not allow intracellular constituents to escape During contraction, however, substances of low molecular weight are rapidly liberated, as if suddenly bursting from the cell

It seems difficult to explain this almost explosive release process except by a rapid shrinking of the cell volume, by which water and solutes are squeezed out of the contracting cell It will be proposed, therefore, that release in platelets exposed to thrombin takes place by extrusion

This effect of contraction may also explain the remarkable formation of long extensions from the cytoplasm in metamorphosing platelets These structures are almost invisible, indeed often too delicate to be seen with the light microscope (KUHNKE 1958) They were nevertheless observed and described by the pioneer investigators HAYEM (1878), BIZZOZERO (1882), SCHIMMELBUSCH (1885) and EBERTH & SCHIMMELBUSCH (1886) Later studies (STUBEL 1914, FERGUSON 1934, BEST, COWAN & MACLEAN 1938 and FONIO 1940) have confirmed these observations

Even more strikingly the cytoplasmic fibrils are displayed in electron micrographs of shadowed preparations of whole platelets, as shown by WOLPERS & RUSKA (1939), BESSIS (1950), HAYDON (1957), HUTTER (1957) and others. The pictures demonstrate these extensions of the cytoplasm to be of remarkably regular appearance They are long and thin fibrils, of almost even thickness except for a short, conical base by which they are attached to the cell A number of fibrils are extended by each platelet, distributed around its margin and pointing radially in all directions The fibrils resist the necessary preparative procedures, including the collection of the cells upon mounting-films and subsequent washing They are evidently somewhat rigid and rather insoluble An example taken from the work of HUTTER (1957) illustrating these characteristics is shown below (Fig 23)

As ment oned [illegible]

alsc [illegible]

fav [illegible] stretches lie in the plane of the section These preparations show that the platelet pseudopodia, at least in their proximal parts, are covered by the surface membrane of the cell

It was pointed out above that the characteristics of the release

reaction may be accounted for by a process of extrusion powered by cellular contraction This also applies to the formation of the platelet pseudopodia These slender structures, whose lengths may be several cell diameters, can hardly be formed except by the forceful ejection of cytoplasmic material in several directions from the main part of the platelet

Several arrangements of the contractile protein within the platelets which allow the development of hydrostatic pressure upon the whole or parts of the cytoplasm during contraction may be possible Probably the simplest mechanical model of the platelet is one in which the major part of the cytoplasm is enclosed within a shell of contractile protein It can be stated without reference to this or other models, however, that the contractile protein must have a definite orientation within the cell, since contractile molecules, in order to have mechanical effects, must have a common direction of contraction

A confirmation of the theory of the formation of platelet pseudopodia by extrusion may be seen in the function of these structures in clot retraction The role of the platelets in this process was convincingly explained by BUDTZ OLSEN (1951) In the words of this author, 'platelets send out fine strands of cytoplasm which join with the cytoplasm of other platelets Subsequently shortening of these strands draws the platelets together, and as

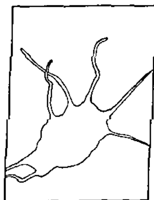


Fig 23 Cytoplasmic extensions of a metamorphosed platelet Drawn from an *electron micrograph* published by HUTTER (1957) The specimen was collected upon the mounting film from platelet rich plasma incubated at 37° C for 10 min Gross clotting was prevented with heparin (0 02 mg per ml of whole blood), microcoagulation with formation of fibrin was observed in addition to the platelet changes

they are firmly attached to the fibrin network the fibrin fibres are also pulled together and the whole clot contracts with a squeezing out of the serum' Direct observational evidence was given for this mechanism, which also satisfactorily explains all known characteristics of the process

The properties which the platelet pseudopodia must possess in order to produce clot retraction by this mechanism are therefore adhesiveness and contractility The latter property may be a direct result of the formation of the platelet extensions by extrusion, because of the molecular orientation established within the pseudopodia during this process

Evidently contractile molecules must be oriented approximately in parallel in order to have a resultant effect when they are contracting The cytoplasmic extensions of the platelets, being the cause of clot retraction, therefore contain contractile molecules of otherwise unspecified nature which are oriented with their direction of contraction along the axis of the pseudopodium Such orientation of asymmetric molecules takes place in streaming An appropriate example is the formation of contractile threads from solutions of actomyosin by extrusion of the protein through a capillary (SZENT GYORGYI 1945) The contractility of the platelet pseudopodia, being dependent on a sufficiently regular orientation of the contractile molecules, may have its origin in the process of extrusion by which they were formed

It is possible on this basis to give a simple description of the mechanism underlying the process of clot retraction It depends, according to BUDTZ OLSEN (1951), on the contractility of the platelet pseudopodia This property results from the formation of these structures by extrusion, which in turn represents an effect of the contraction of the platelet Some well known characteristics of the process of clot retraction are particularly easy to explain by this mechanism Thus it is self evident that clot retraction can only take place in the presence of blood platelets, as was first pointed out by HAYEM (1896) and repeatedly confirmed by later investigators (LESOURD & PAGNIEZ 1907, OPITZ & SCHOBER 1923, TONIO 1923 BUDTZ OLSEN 1951, and others) It is also obvious why platelets must be structurally intact if they are to be able to produce retraction The long platelet fibrils, with the necessary orientation of constituent molecules, can only be formed by extru-

sion from whole cells whose contractile properties are undamaged This explains why extracts of platelets are found to be without effect, whatever their method of preparation

Other characteristics of clot retraction are explained by the *properties of the contractile system of platelets* Thus, clot retraction has been found to require the presence of calcium ions (LESOURD & PAGNIEZ 1913, BUDTZ-OLSEN 1951, LUSCHER 1956, ZUCKER & BORRELLI 1959), whose role in the initiation of con traction has been demonstrated previously in the present work

Clot retraction is also markedly sensitive to an increase in hydrogen ion concentration, and has been shown to be inhibited at pH values of about 6 0 (ZUCKER & BORRELLI 1959, CORN, JACKSON & CONLEY 1959) This effect can be explained from the influence of the hydrogen ion concentration on platelet contraction As shown by the following experiment (Fig 24) the extent of release is markedly reduced at pH 6 0 and release is completely inhibited at pH 5 5 According to the extrusion theory the formation of platelet pseudopodia, which are responsible for clot retraction, is similarly affected by the pH of the system Clot retraction is therefore prevented

It follows from the present conclusions that it is the formation of the contractile platelet fibrils, and not their subsequent short-

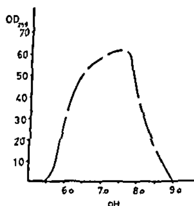


Fig 24 Effect of pH on the extent of the release reaction Samples of platelets (0 025 g) were incubated at 37° C with 0 5 mg of thrombin in 1 5 ml of 0 15 M NaCl for 10 min The hydrogen ion concentration was adjusted with acetate (pH 5 5), phosphate (pH 6 0–7 5) and Tris buffers (pH 8 0–9 0) in 0 03 M final concentrations Incubation was terminated by centrifugation and released adenine nucleotides were determined in 1 0 ml of the platelet free supernatant solutions

ening, which has the character of muscle-like contraction Their shortening clearly takes place by a different process, because ATP must be rapidly lost by diffusion from these exposed structures It is in accord with this view that the velocity of retraction is very low, pointing to a process which differs from the ATP dependent shortening of contractile protein fibres

As described above, the morphological characteristics and the contractility of the platelet pseudopodia can be explained from their formation by extrusion Clearly these structures are also adhesive, since their contraction would be without effect on the clot unless adhesions are formed between the platelet fibrils or between the fibrils and the fibrin threads This adhesiveness may be considered as the cause of the irreversible aggregation of platelets in viscous metamorphosis, since it is by their long pseudopodia that metamorphosed platelets will come into mutual contact The firmness of the platelet aggregates, expressed by their complete resistance towards resuspension of the cells, attests to the strength of the bonds formed

CHAPTER 9

General Summary

The present work started with an inquiry into the mechanism by which 5-hydroxytryptamine (Serotonin) is liberated from blood platelets by thrombin Few facts existed concerning the nature of this effect, although it was assumed from characteristics of the enzyme that a proteolytic reaction with a substrate in the platelet surface is involved The identity of this substrate and the connection between its hydrolysis and release of 5-hydroxytryptamine were unknown

Apart from its ability to cause release of 5-hydroxytryptamine, thrombin was known to produce viscous metamorphosis in platelets The mechanism of action of thrombin in the latter process had not been established It was also unknown whether release of 5-hydroxytryptamine and viscous metamorphosis were independent phenomena produced by this enzyme, or whether they had further mechanisms in common

In studies carried out on these questions and on problems encountered in the course of the investigation the occurrence of release of intracellular constituents from platelets exposed to thrombin represented the central phenomenon It could be shown that a number of platelet substances are released in parallel, indicating a fundamental effect on platelet structure Adenine nucleotides found to take part in release were employed as indicator substances in following the process

Thrombin-catalyzed release takes place without lysis of the cells It is therefore a specific platelet phenomenon, which has been described as *the release reaction of the platelets* in the present work

As part of studies aimed towards identification of the substrate for thrombin in the release reaction the proteins of platelets were subjected to extraction and fractionation Special attention was paid to fibrinogen, known from previous studies to be present in platelet extracts Fibrinogen was found to represent about 4 % of

the total protein of platelets, suggesting that it occurs intracellularly rather than externally adsorbed upon the cells This was confirmed by experiments with trypsin, to which platelet fibrinogen was found to be resistant

Platelets incubated with trypsin and subsequently exposed to calcium ions were observed to liberate intracellular constituents and undergo irreversible aggregation, in a process indistinguishable from the release reaction In this release platelet fibrinogen was found to be specifically liberated The effects could be reproduced with thrombin, when incubations with this enzyme were carried out at low temperature in the presence of a calcium complexing agent This resistance of platelet fibrinogen towards trypsin and thrombin indicates that it does not function as a substrate for the proteolytic enzymes in the release reaction The substrate on which thrombin and trypsin are acting in initiating release is therefore a different and as yet unidentified protein

In the work on extraction of fibrinogen a second protein fraction of platelets, which forms about 20 % of their total protein, was isolated This protein was identified as actomyosin like and contractile The observation represents independent confirmation of results obtained previously by others

Kinetic studies were carried out on the release reaction in order to define its nature more closely At relatively low temperatures (about 15° C) release was found to have the characteristics of a multi-stage process. It was confirmed that thrombin represents the first stage in this sequence Evidence was obtained which indicated that it is followed by a reaction which requires calcium Contraction of the platelet was found to represent the last stage in the release reaction

Studies were carried out on the functions of the different steps in the release reaction The role of the thrombin catalyzed step, assumed to be a proteolytic process in the surface membrane of the platelet, appeared to be a breakdown of permeability barriers which allows subsequent penetration of calcium ions. These ions, appearing intracellularly, initiate contraction

The fact that calcium is a normal constituent of platelets, which nevertheless do not contract unless exposed to thrombin, indicates that platelet calcium is either not ionized or is prevented by diffusion barriers from interacting with the contraction system There

was evidence however, that intracellular calcium is activated and takes part in the release reaction when the platelets are exposed to thrombin

The role of calcium ions in platelet contraction was provisionally explained as analogous to the function of these ions in abolishing relaxing factor activity in striated muscle The demonstration of relaxing factor in platelets, however, was not attempted

An investigation of viscous metamorphosis was carried out in order to define its possible connections with the release reaction The studies showed that viscous metamorphosis and release are parallel phenomena, dependent on the same fundamental mechanisms in the platelets Like release, viscous metamorphosis is therefore an effect of platelet contraction

Known alterations in platelet structure during viscous metamorphosis must be taken into account in order to explain the mechanical effects of platelet contraction The correlation of these alterations with release suggests a process of extrusion, in which water and soluble constituents of low molecular weight as well as fibrinogen are forcibly ejected It is implied that hydrostatic pressure develops upon the cytoplasm in contracting platelets This also explains the formation of platelet pseudopodia

According to previous theory accepted in the present work platelet pseudopodia formed in viscous metamorphosis are the active elements in clot retraction It is implied in this theory that the structures possess contractility, which property may be established by molecular orientation in the streaming cytoplasm during *extrusion of the pseudopodia Indirectly clot retraction* thus becomes dependent on platelet contraction However the subsequent shortening of platelet pseudopodia must be different from musclelike contraction

A second property consisting in strong adhesiveness is also demonstrated by the platelet pseudopodia through their role in clot retraction This property may be the cause of the irreversible aggregation which is a prominent feature of viscous metamorphosis

In present theory agreement has not yet been reached with respect to the mechanism by which platelets acquire the adhesiveness responsible for initial aggregation into a platelet plug It is a central question in this connection whether adhesiveness may be

obtained *in vivo* without the intervention of thrombin The present studies can throw no further light on this problem

Once formed, however, the platelet plug undergoes secondary alterations by which it is rendered more solid, adherent, and compact These alterations are essential In their absence hemostasis fails to be established The transformation of the loosely aggregated mass of platelets into a hemostatically effective platelet plug has been shown to be an effect of thrombin Its mechanism therefore corresponds to the reaction sequence studied in the present work

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Cellular localization of brain monoamines

BY

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and NILS-ÅKE HILLARP

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FROM THE DEPARTMENT OF PHARMACOLOGY UNIVERSITY OF GOTEBORG AND THE DEPARTMENT OF HISTOLOGY UNIVERSITY OF LUND SWEDEN

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CONTENTS

With the use of a fluorescence method for histochemical demonstration of catecholamines evidence has been obtained for the view that NA[1] in the hypothalamus is accumulated in what appears to be synaptic nerve terminals (CARLSSON *et al* 1962) This amine may consequently serve as a synaptic transmitter in the brain

Further work on the cellular localization of monoamines in the brain is reported in this paper

[1] *Abbreviations* DA = 3 4-dihydroxyphenylethylamine (dopamine), NA = noradrenaline A = adrenaline dopa = 3 4-dihydroxyphenylalanine 5-HT = 5-hydroxytryptamine

MATERIAL AND METHODS

The animals were killed by decapitation (mouse, rat) or by an i v injection of air (rabbit) The brain was immediately taken out and various parts were excised and frozen in propane cooled by liquid nitrogen After freeze drying at —35° for 8 to 10 days the preparations were treated with formaldehyde gas (from paraformaldehyde) at +80° for 1 hr During this treatment the catecholamines and 5 HT — without diffusion — condense with formaldehyde to intensely fluorescent products which are not extracted by hot paraffin or xylene The preparations were then infiltrated *in vacuo* with paraffin at +60° for 10 min Sections (8 μ) were mounted in Entellan (Merck) The fluorescence is preserved fairly well for at least 2 days in this medium — The details of the procedure and the equipment for fluorescence microscopy are described in another paper (Falck 1962)

So far only the hypothalamus and caudate nucleus have been studied more extensively Here the fluorescence method gives constant and reproducible results In order to localize the fluorescent material the sections were stained with gallocyanin chromalum or toluidin blue after the fluorescence examination

Catecholamines and 5 HT were determined spectrophotofluorimetrically (see Carlsson and Lindqvist 1962)

Reserpine and α methyl m tyrosine were kindly donated by Ciba Ltd , Basle and Dr K C Mezey of Merck Sharp and Dohme Research Laboratories Rahway New Jersey, respectively The m tyrosine used was obtained from Sigma Chemical Company The solvents for the drugs were given to control animals

RESULTS

I. *Fluorescent structures in normal brain*

Several different types of structures in the central nervous system of normal animals develop a clearly observable green to yellow-green or green-yellow to yellow fluorescence, characteristic of certain catecholamines and tryptamines, respectively This fluorescence appears only under conditions which are necessary to obtain intensely fluorescent products of these amines (*cf* FALCK 1962) So far no other fluorescence has been found to develop and the background fluoresces very faintly Thus an excellent contrast is obtained against an essentially dark background This is true especially if the sections are examined with a suitable dark field condensor which prevents most of the exciting light to reach the objective and thus also permits the use of exciting light of high intensity

So far no fluorescence at all of this kind — besides the general background — has been found to develop in brains from reserpinized animals (see Section II) This and other results obtained in extensive studies of model systems and tissues (FALCK *et al* 1962, FALCK and TORP 1962, FALCK 1962 and unpublished results) show that the fluorescence method used has a high specificity for certain catecholamines and tryptamines The evidence hitherto obtained seems to show that other biogenic compounds under the conditions used either cannot develop a fluorescence in the green to yellow range which is sufficiently strong to interfere with the localization of tissue monoamines or — if so — exist in too small concentrations in normal tissues to be of significance in this respect.

Some autofluorescent material is present here and there in the brain It does not seriously interfere with the amine localization, however it usually fluoresces in b[illegible]
and never in r[illegible]
reserpine or w[illegible]
water content

Another important point is that the principal features of the reaction between catecholamines (and tryptamines) and formaldehyde, including the chemical requirements and molecular structures necessary for the formation of intensely fluorescent compounds, are fairly well understood (FALCK *et al* 1962 CORRODI *et al* 1962) The fluorescence method is thus no empirical method with an obscure chemical basis and can consequently be used with much more confidence than otherwise would be possible

Varicose fibres with green to yellow green fluorescence

An intense green to yellow green fluorescence which stands out brilliantly against an essentially dark background develops in four circumscribed and bilaterally symmetric areas within the *hypothalamus* 1) a large area in the preoptic region just below (and partly above) the lateral part of the anterior commissure, 2) the supraoptic nuclei (Fig 1), 3) the paraventricular nuclei (Fig 2), 4) an area in the walls of the third ventricle (the periventricular nuclei) within the anterior hypothalamus (Fig 3) The fluorescent products are localized to fine fibres running between and enclosing the nerve cells which themselves — like the glia cells — show no fluorescence (Fig 4, 6, 8 and 9) Because of the fineness of the fibres, their dense accumulation and tortuous course in these nuclei they do not show up very distinctly in microphotos The fibres are readily observable in the microscope however, especially after a low dose of reserpine when the fluorescent material is reduced (Section II) Similar fluorescent fibres are found scattered in almost every part of the hypothalamus (Fig 4 and 7) They are more scarce in the posterior region and entirely missing in the suprachiasmatic nuclei (Fig 10) and in the optic nerves, chiasma (Fig 1), and tracts — More dense accumulations of fibres are found in two areas which are not so distinctly delimited in the preoptic region just above the optic nerves and a fairly large area just posterior to the paraventricular nuclei

That part of the fibres which contains fluorescent material seems to be short Fluorescent fibres longer than 0 1 mm have been seen only occasionally in sections cut through different planes The fibres — although finer — have the same characteristic appearance as the adrenergic nerve terminals in peripheral tissues with small intensely fluorescent enlargements dispersed at irregular intervals (Fig 4, 7, 8, and 9) The thickness of these varicosities usually is 0 3 to 0 8 μ and seldom exceeds 1 μ Between the enlargements the fibres usually are so thin that they are just visible or partly cannot be seen at all Many fibres seem to be submicroscopic and are observable only because of the intense fluorescence of the varicosities — In the anterior hypothalamus some much thicker fibres may be found (Fig 5)

TABLE I *The brains from 20 rats (250—300 g body wt) were used The anterior part of the hypothalamus included the paraventricular and most of the supraoptic nuclei The posterior hypothalamus was the part posterior to the hypophysial stalk*

Part of the brain	Wet weight mg	NA content μg g
Amygdala	170	0 35
Preoptic region	356	2 2
Anterior hypothalamus	348	1 9
Intermediate	317	2 3
Posterior	333	1 2



Fig 1* Abundant intensely fluorescent material in the supraoptic nucleus No fluorescence in the optic chiasma (left) Intense fluorescence of the adrenergic nerves around the artery at the basal surface of the brain (below the chiasma to the left) The internal elastic membrane of the artery shows auto-fluorescence Magnification 150 ×

The fibres are not ordinary adrenergic nerves to blood vessels since bilateral cervical sympathectomy does not cause them to disappear Furthermore so far we have found no adrenergic nerve supply to the vessels *within* the hypothalamus of the mouse and the rat whereas such nerves are constantly and easily detectable in the vessels at the basal surface of the brain. (A scarce adrenergic nerve supply was found in the largest arteries penetrating the brain tissue of the cat)

The fibres seem to run to nerve cells which are partly enclosed by them (Fig 7 9 and where they partly seem to terminate with a small knob In the nuclei with dense fibre accumulations the fibres appear to form real baskets around the nerve cells (partly seen in Fig 6) In areas where the nerve cells do not lie so closely packed it may be clearly observed that many of the fibres in each section (8 μ) after a short run between the cells become intimately associated each with one nerve cell the fibre — as it seems —

All figures are fluorescence micrographs of frontal sections (8 μ) through the hypothalamus of normal rat The freeze-dried tissue was treated as described in Material and Methods to obtain intensely fluorescent products (white in the micrographs) from the catecholamines and tryptamines.

Varicose fibres with green to yellow green fluorescence

An intense green to yellow green fluorescence which stands out brilliantly against an essentially dark background develops in four circumscribed and bilaterally symmetric areas within the *hypothalamus* 1) a large area in the *preoptic region just below* (and partly above) the lateral part of the anterior commissure, 2) the supraoptic nuclei (Fig 1), 3) the paraventricular nuclei (Fig 2), 4) an area in the walls of the third *ventricle (the periventricular* nuclei) within the anterior hypothalamus (Fig 3) The fluorescent products are localized to fine fibres running between and enclosing the nerve cells which themselves — like the glia cells — show no fluorescence (Fig 4, 6 8, and 9) *Because of the fineness of the fibres, their dense accumulation and tortuous course in these nuclei* they do not show up very distinctly in micro photos The fibres are readily observable in the microscope however, especially after a low dose of reserpine when the fluorescent *material is reduced (Section* II) Similar fluorescent fibres are found scattered in almost every part of the hypothalamus (Fig 4 and 7) They are more scarce in the posterior region and entirely missing in the suprachiasmatic nuclei (Fig 10) and in the optic nerves chiasma (Fig 1), and tracts — More dense accumulations of fibres *are found in two* areas which are not so distinctly delimited in the preoptic region just above the optic nerves and a fairly large area just posterior to the paraventricular nuclei

That part of the fibres which contains fluorescent material seems to be short Fluorescent fibres longer than 0 1 mm have been seen only occasionally in sections cut through different planes The fibres — although finer — have the same characteristic appearance as the adrenergic nerve terminals in peri *pheral tissues with small intensely* fluorescent enlargements dispersed at irre gular intervals (Fig 4, 7, 8, and 9) The thickness of these varicosities usually is 0 3 to 0 8 μ and seldom exceeds 1 μ Between the enlargements the fibres usually are so thin that they are just visible or partly cannot be seen at all Many *fibres seem to* be submicroscopic and are observable only because of the intense fluorescence of the varicosities — In the anterior hypothalamus some much thicker fibres may be found (Fig 5)

TABLE I *The brains from 20 rats (250—300 g body wt) were used The anterior part of the hypothalamus included the paraventricular and most of the supraoptic nuclei The posterior hypothalamus was the part posterior to the hypophysial stalk*

Part of the brain	Wet weight mg	NA content μg g
Amygdala	170	0 35
Preoptic region	356	2 2
Anterior hypothalamus	348	1 9
Intermediate	317	2 3
Posterior	333	1 2



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Fig 2 Abundant, intensely fluorescent material in the paraventricular nucleus Magnification: 150 x.

approaches a cell and then becomes directly superimposed and terminates on the perikaryon. For these and other reasons (see Discussion) it seems probable that the fluorescent fibres represent the terminal parts of axons forming synaptic contacts with nerve cells

The fluorescence reaction and the properties of the fluorescent material have been examined in the same ways as those used in studies on model systems and adrenergic nerves in peripheral tissues (see FALCK 1962). In all



Fig 3 Abundant intensely fluorescent material in the periventricular nucleus of the anterior hypothalamus. Magnification 150 ×

respects the hypothalamic and adrenergic "terminals" show the same characteristics This strongly supports the view that the fluorescence developing in the hypothalamic fibres is due *to* the presence of a primary catecholamine Further evidence for this view *was obtained* in experiments on the effects of some drugs described in sections II and III

Similar fluorescent fibres are present *in other* parts of the central nervous system They are scarce in the *cerebral cortex* (only a few areas have been examined so far) and only very scattered fibres are seen in the *cerebellum* In contrast more or less *dense* accumulations exist *in* certain regions of the *pons* and the *medulla oblongata* A *small* number of fibres are also present in the *spinal cord* However, these findings will be described in another paper

Median eminence

A yellow-green fluorescence develops also in the median eminence Here, however the fluorescent *material is* not present in distinct fibres but exists as

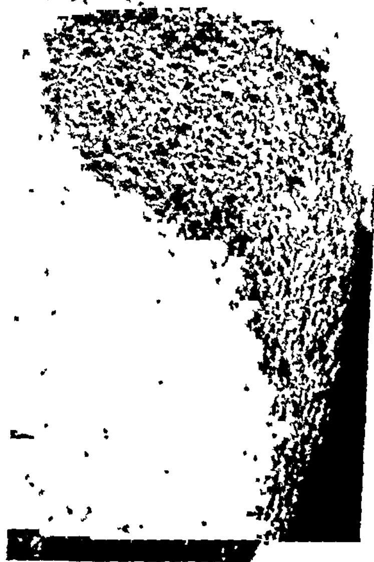


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Fig 5 Thick fluorescent fibres with varicosities in the anterior hypothalamus Magnification 250 x

Fluorescent nerve cells

A group of small nerve cells which develop a fairly weak green fluorescence are present in the lateral walls of the third ventricle above the median eminence (the arcuate nuclei) A stronger green to yellow green fluorescence is obtained in the bodies and larger processes of big nerve cells which — although fairly scattered — form a large group in the posterior hypothalamus and pons Fluorescent nerve cells are found also in other regions of the central nervous system (e g the medulla oblongata) but no detailed studies of their localization and reaction to drugs have been made

Smooth fibres with yellowish fluorescence

In several circumscribed areas there are fairly dense accumulations of fibres which develop a more yellowish fluorescence. The colour of the emitted light varies from green yellow to yellow The fibres are very fine — often just visible — and seem to be completely smooth However, sometimes the fibres are more distinctly seen — especially after reduction of the fluorescence by



Fig 4 The wall of the third ventricle (right) in the anterior hypothalamus The fluorescent material is present in fine fibres with varicosities which fluoresce intensely Magnification 375 ×

a dense accumulation which forms a continuous and distinct zone with an intense fluorescence This zone is localized to the superficial part of the nervous tissue where the primary plexus of the hypophysial portal system arises It begins in the rostral part of the median eminence and continues down into the hypophysial stalk The outer borderline is quite sharp but somewhat irregular on account of indentations caused by the portal vessels and, in fact, coincides with the external surface of the nervous tissue Towards the interior parts of the brain the fluorescence fades fairly diffusely, and irregular, faintly fluorescent stripes radiate towards the infundibular recess of the third ventricle It has not yet been possible to decide in what cell structures the fluorescent material is localized

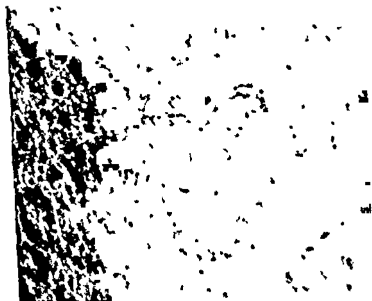


Fig 7 Fine fluorescent fibres with varicosities in the area lateral to the paraventricular nucleus (left) Magnification 250×

best optical conditions available one gets the impression that the fluorescent material is at least partly localized to very fine fibres which are closely packed Their nature is unknown — The areas with this diffuse fluorescence are distinct and fairly circumscribed The rostral areas which are very large, are continuous with smaller areas above the preoptic region and anterior hypothalamus and also with the amygdala which as a whole shows the same general appearance A similar fluorescence develops also in the caudate nuclei (see Section V)

II *The effect of reserpine*

To study the effect of reserpine white rats (200—250 g body wt.) of our own stock were divided in groups of 5 to 7 animals Reserpine was administered subcutaneously and the animals were killed after various time intervals The hypothalamus and adjoining brain parts were examined

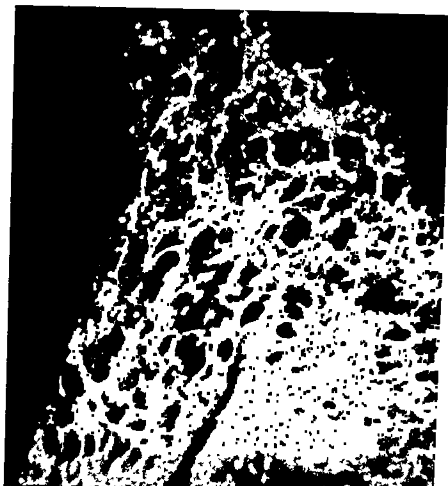


Fig 6 Supraoptic nucleus The fluorescent material can partly be seen to be localized to fine varicose fibres enclosing the nerve cells which are non-fluorescent (dark in the micrograph). Magnification. 375 x.

drugs (see below) — and then several of them appear to have small enlargements and a synaptic arrangement Their nature has nott been elucidated but they may well be nerve fibres. — A closer anatomical localization of the areas has not been made as yet No circumscribed accumulations of such fibres are present within the hypothalamus but several occur in regions surrounding the preoptic area anteriorly, laterally, and dorsally. Such fibres are also accumulated in the medial part of the amygdala

Areas with diffuse greenish fluorescence

There is still another type of fluorescence, i a in the parts of the brain which surround the preoptic region and anterior hypothalamus The fluorescence is fairly weak, green to yellow-green, and has a diffuse character so that no distinct fluorescent structures can be observed When it is studied under the



Fig 8 B

in the median eminence had been markedly reduced to a thin weakly fluorescent superficial zone The bodies of the posterior big nerve cells fluoresced in green with reduced intensity and the processes fluoresced only *faintly The diffuse* fluorescence of the amygdala etc and the fluorescence of the fine smooth fibres were decreased very considerably — In two of the six animals the fluorescence reduction was still more marked in all the *examined* regions

Administration of 0 5 mg/kg caused almost the same marked fluorescence reduction as that described above No fluorescence at all was observed in the supraoptic nuclei and the fluorescent material *in the* median eminence had

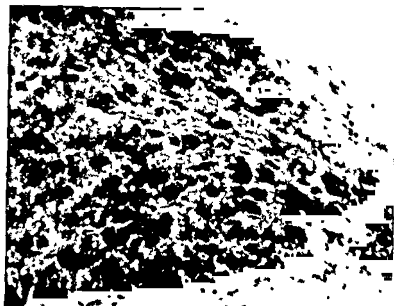


Fig 8 A

Fig 8 and 9 Paraventricular nucleus The fluorescent material can partly be seen to be localized to fine varicose fibres enclosing the nerve cells which are non fluorescent (dark in the micrographs) Magnification 250 × (8 A) and 375 × (8 B and 9)

The effect of varying doses of reserpine at a fixed time interval (24 hours)

After a single injection of 5 mg/kg no fluorescence could be developed except in the fluorescent nerve cells Their perikarya fluoresced very faintly (not at all in some animals) and the processes could not be seen — The findings were the same in mice treated with a dose of 25 mg/kg or with a dose of 1 mg/kg administered on each of four consecutive days

After administration of 1 5 mg/kg no fluorescence at all appeared in the supraoptic nuclei However, several weakly fluorescent fibres with typical enlargements emitting more intense green light were present in the paraventricular and periventricular nuclei The large area in the preoptic region showed a distinctly weaker fluorescence than normally but contained abundant fine fibres with typical enlargements which stood out very distinctly against the dark background In the region of the anterior hypothalamus just above the optic tracts several thick, varicose fibres with a fairly strong fluorescence were present The denser accumulation of varicose fibres just behind the paraventricular nuclei showed up more distinctly than in normal brains although the fibres fluoresced weakly This was due to a marked reduction of fluorescent fibres in the surrounding areas The reduction was considerable also in the other parts of the hypothalamus The accumulation of fluorescent material



Fig 10 Suprachiasmatic neucleus There are no fluorescent varicose fibres in this nucleus but many in the area above it, especially in the wall of the third ventricle (right) Magnification 100 x

most affected Fluorescent material was partly lacking in the medial and dorsal parts of the supraoptic nuclei and it was markedly diminished in the other parts where its localization in weakly to rather strongly fluorescent fibres possessing abundant varicosities and enclosing the nerve cells could now be seen much more distinctly than in the brains from normal animals The fluorescent material in the median eminence was decreased almost as markedly as in the preceding group In contrast to the big posterior cells which seemed largely unaffected, the small nerve cells in the arcuate nuclei displayed no or very weak fluorescence A more or less distinct — although not marked — reduction was found in all other areas — Also the adrenergic nerves in the vessels at the base were affected In one of the animals they were non fluorescent and in the others they fluoresced with a fairly low intensity

The effect of a fixed dose of reserpine (5 mg/kg) at varying time intervals

Also in these experiments the supraoptic nuclei and median eminence were most readily affected In the median eminence of five of the animals killed after 2 hours fluorescence was completely lacking In the remaining two animals it was very weak All the animals showed a marked reduction in the



Fig 9

also been extensively diminished as in the preceding group — Just as after the higher doses the adrenergic nerves in the walls of the vessels at the basal surface of the brain did not show up at all

Also the smallest dose used — 0 15 mg/kg — caused a considerable reduction in some of the areas The supraoptic nuclei and median eminence were



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supraoptic nuclei where only scarce weakly fluorescent varicose fibres persisted In contrast many fibres with good fluorescence were still present in the paraventricular nuclei and especially in the preoptic area Nor were marked changes observed in the other regions with varicose fibres The greenish fluorescence of the small and large nerve cells was clearly reduced or had disappeared completely however The areas with diffuse fluorescence and smooth fluorescent fibres showed reduced intensity Many of the very fine fibres could now be seen more distinctly and appeared to be varicose and to enclose nerve cells in a way suggesting a synaptic arrangement — The adrenergic nerves around the vessels at the base showed a more or less marked reduction of fluorescence intensity

After 4 hours only scarce varicose fibres with a weak fluorescence persisted in the paraventricular nuclei and scattered in other parts of the hypothalamus but many fibres in the area of the preoptic region still fluoresced fairly well The greenish fluorescence of the median eminence and the small and big nerve cells had disappeared The areas with diffuse fluorescence and fine smooth fluorescent fibres were mostly barely visible — The adrenergic nerves around the vessels at the base showed no or very weak fluorescence

After 8 hours the normal fluorescence had disappeared practically completely everywhere The area in the preoptic region was almost the only place where some varicose fibres with weak fluorescence still were found — A finding of as yet unknown significance was the appearance of a granular material with a fairly strong fluorescence in yellow orange orange or yellow brown It was localized to the bodies and partly also to the processes of nerve cells especially in those areas which normally have abundant fluorescent fibres There is thus an interesting possibility that it has something to do with the altered monoamine metabolism in reserpinized animals The material itself might be an amine metabolite for instance These possibilities are under study

In animals killed 24 and 48 hours after administration of reserpine no fluorescence could be developed except a very weak one in the bodies of the big posterior nerve cells and the cells in the arcuate nuclei After 3 days these cells partly began to recover a more distinct greenish fluorescence and some of the processes of the big cells could be seen again Some weakly fluorescent fibres with typical enlargements reappeared in the median eminence in the walls of the third ventricle above it and — in two of the animals — also in the preoptic area The superficial zone of the median eminence also began to recover and now had developed a fairly weak and diffuse fluorescence No recovery was found in any other areas (or in the vessels at the base)

After 5 days the small and big nerve cells seemed largely to have recovered but the fluorescence in the superficial zone of the median eminence was far below the normal level At this time weakly fluorescent fibres with typical varicosities had began to reappear in all the places where such fibres are present normally (including the vessels at the base) The areas with diffuse fluorescence and fine smooth fibres also had partly recovered

III. *The effect of m-tyrosine and α-methyl-m-tyrosine*

When m tyrosine is administered intraperitoneally to mice in three doses of 400 mg/kg each with 2 hours interval and the animals are killed 2 hours after the last injection the DA and NA content in the brain is decreased below 10 per cent of the normal level, but the 5-HT content is practically unaffected (CARLSSON and LINDQVIST 1962, and unpublished results)

Ten mice were treated with *m-tyrosine in this way* and the hypothalamus and adjacent brain parts were examined The fluorescent varicose fibres had disappeared in all areas The superficial zone in the median eminence had lost most or practically all of its fluorescence The small cells in the arcuate nuclei and the big posterior nerve cells fluoresced weakly or not at all The areas with diffuse fluorescence could still be identified but the intensity of their fluorescence was not much above the general background. In contrast to this the areas with fine smooth fibres seemed to be largely unaffected and emitted a more yellowish light — A small general increase in the background fluorescence (greenish) was observed in all the animals This probably was *due to m tyrosine still being present in the brain since this amino acid* (and also α methyl m tyrosine) on condensation with formaldehyde under the conditions used develops a green to yellow green fluorescence This view is supported by the finding that such an increase was not present 24 hours after the administration of α-methyl m tyrosine (see below)

The same very marked reduction of the fluorescence was found in two animals which were killed 2 hours after a single subcutaneous injection of 800 mg/kg In five animals killed 4 1/2 hours after a single intraperitoneal injection of 400 mg/kg some distict and varicose fibres with a weak fluorescence were present especially in those regions where they normally occur in abundance The fluorescent zone in the median eminence was very markedly reduced also in these animals but the big posterior nerve cells seemed less affected

Seven mice were killed 24 hours after an intraperitoneal injection of α-methyl m tyrosine (400 mg/kg) In three of them the dose was divided into two injections made with an interval of 3 hours This treatment reduces the brain NA to a level below 10 per cent of normal without any significant depletion of the 5-HT and with but slight decrease in the DA (HESS, OZAKI and UDENFRIEND 1960, BRODIE, MAICKEL and WESTERMAN 1961, CARLSSON and LINDQVIST 1962) The fluorescence findings were practically identical with those obtained in the animals treated with 3×400 mg/kg of m tyrosine The superficial zone of the median eminence had been reduced to a thin band which however, still fluoresced with fairly high intensity in contrast to the finding in the m-tyrosine animals The areas with diffuse fluorescence also seemed less affected No increase in the general background fluorescence had occured

In four mice killed 24 hours after a single low dose (50 mg/kg) of α methyl m tyrosine a more or less marked decrease in the fluorescence of the

varicose fibres and the big posterior cells had occured The fluorescent zone in the median eminence and the areas with diffuse fluorescence appeared unaffected, however

IV *The effect of nialamide in reserpinized mouse*

Nialamide (500 mg/kg) was injected intraperitoneally in ten mice 24 hours after the administration of reserpine (25 mg/kg) The animals were killed 6 hours later and the hypothalamus was examined Reserpine causes a depletion of the monoamine stores in the brain but nialamide brings about a considerable reaccumulation of 5 HT (almost up to normal levels) without affecting the catecholamine levels (CARLSSON, LINDQVIST and MAGNUSSON 1960 and unpublished data)

The distinct varicose fibres normally exhibiting a green to yellow green fluorescence and the median eminence had not acquired any fluorescence at all The small cells in the arcuate nuclei and the big posterior nerve cells showed no or very faint greenish fluorescence Unfortunately, the fluorescent areas outside the hypothalamus were not included in the preparations

The most interesting observation is, however, that nerve cells and fibres normally without any obvious fluorescence now exhibited a more or less intense fluorescence of a yellow colour, suggesting the presence of a tryptamine Fairly big nerve cells with fluorescent perikarya and processes were localized to the posterior hypothalamus and pons, and fluorescent fibres both very fine smooth and thicker varicose ones, were found in these regions and in the most lateral parts of the hypothalamus — The findings might suggest that 5 HT had accumulated in these structures Further studies are necessary however, to determine the cellular localization of this amine

V *Fluorescence of the caudate nucleus*

No distinct fluorescent fibres or nerve cells have been found in the caudate nucleus of the rat and the mouse But the nucleus as a whole develops a marked yellow green to green fluorescence which stands out well against the dark brain regions surrounding it The fluorescence is quite diffuse and somewhat uneven small irregular areas with somewhat higher intensity are present everywhere In contrast to this the nerve cell bodies and fibre bundles fluoresce very faintly or not at all

Reserpine (rat 5 mg/kg, mouse 25 mg/kg) caused a so complete disappearance of this diffuse greenish fluorescence within 24 hours that the caudate nucleus could not be distinguished from the surrounding regions any longer Most of the fluorescence had been lost already after 2 hours

Meta tyrosine (mouse, 3×400 mg/kg see Section III) caused a more or

Table II The table summari es the changes of the d ffuse fluorescence in the caudate nucleus of mice after administration of drugs (doses etc see Section 3) The data on the changes of the amine content have been obtained from extensive experiments (mos ly unpublished) on mice and other animals made in the Department of Pharmacology Goteborg It must be noted however that the amine changes reported above have been inferred from the changes found in whole brains of mice

Treatment of the animals	Caudate Nucleus Fluorescence	DA content	5 HT content
Reserp ne	Lost	Lost	Lost
Meta tyrosine	More or less marked reduction	Lost	Largely unchanged
a methyl m tyrosine	Somewhat reduced	Somewhat reduced	Largely unchanged
Nialamide	No obvious increase	Slight or no increase	Increased
L-dopa	No obvious increase	Small increase	No increase
N alam de + L-dopa	Very marked increase	Very marked increase	Increased
Reserpine + nialam de	Almost completely lost	Almost completely lost	Normal or slight decrease

less marked reduction but in all animals the nucleus still showed a definite greenish fluorescence In contrast to this most of the fluorescence persisted after the administration of *a methyl m tyrosine (mouse, 400 mg/kg*, see Section III)

No obvious increase of the fluorescence was observed after administration of *nialamide* (mouse 500 mg/kg i p 6 hours) or *L-dopa* (mouse, 100 mg/kg subcut 1 hour) However, a very marked increase was obtained when the dopa was given 5 hours after the injection of nialamide The nerve cells and nerve bundles were still practically non fluorescent but the capillary walls exhibited a fairly intense yellow green fluorescence, probably due to an accumulation of dopa (unpublished data) The regions surrounding the caudate nucleus showed no certain increase of the background fluorescence and the borderline was just as sharp as in normal animals.

In reserpinized mice (25 mg/kg 24 hours) *nialamide* (500 mg/kg 6 hours) did not cause any obvious increase in the very faint diffuse fluorescence

Discussion

The dominating structure which develops fluorescence in the hypothalamus is the fine varicose fibres assumed to be the terminal parts of axons forming

varicose fibres and the big posterior cells had occured The fluorescent zone in the median eminence and the areas with diffuse fluorescence appeared unaffected, however

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this amine — although present in the whole neuron — is accumulated and stored in very high concentrations in the 'terminals" of the adrenergic nerves. This accumulation has recently been directly demonstrated (FALCK and TORP 1962) The fluorescent varicose fibres in the hypothalamus have the same charateristic appearance and must — as judged from the fluorescence intensity — accumulate and store similar very high concentrations of NA as the "terminals" in peripheral tissues Besides the morphological evidence (section I) there is thus also biochemical evidence for the view that the fluorescent fibres represent the terminal parts of axons The very high accumulation of NA in these terminal parts is just what would be expected if they are 'terminals" belonging to adrenergic neurons in the brain

There are thus reasons to believe that adrenergic neurons exist in the central nervous system. From this point of view it is highly interesting that some nerve cells in the hypothalamus and also other regions develop a fluorescence in the cell bodies and larger processes which suggest the presence of a catecholamine in low concentrations The findings that this fluorescence may be caused to disappear through administration of reserpine, m tyrosine and α-methyl m-tyrosine strongly support this view These nerve cells may thus represent the cell bodies of adrenergic neurons

The fluorescent zone in the median eminence is puzzling The results obtained so far indicate that a catecholamine is accumulated here but its cellular localization has not been revealed However, the intimate relation of the zone to the hypophysial portal system suggests a rôle in the humoral regulation of the pituitary functions Preliminary examinations (J HÄGGENDAL, unpublished data) of the monoamines in the median eminence suggest a high DA content. The finding that much of the fluorescence still persisted after α-methyl m-tyrosine — in contrast to m-tyrosine — also might suggest the presence of this amine (*cf* CARLSSON and LINDQVIST 1962)

Like NA, 5-HT easily gives an intensely fluorescent condensation product with formaldehyde under the conditions used and the fluorescence method readily demonstrates the 5-HT present in the mast cells of some species (FALCK 1962) However it has proved difficult to visualize the brain stores of this amine This might be due to a localization to submicroscopic structures which are not closely packed or to a so widespread distribution that the concentration does not reach the limit of detection The very fine smooth fibres with a more yellowish fluorescence may well contain 5 HT, however The colour of the emitted light and the findings that the fluorescence disappears after administration of reserpine — but not m tyrosine or α-methyl m tyrosine — favour this view

The diffuse fluorescence of the circumscribed and more or less continuous areas including the amygdala, surrounding the anterior hypothalamus and preoptic region seems to be due to the presence of a catecholamine, possibly both NA and DA. The characteristics of the fluorescence reaction, the properties of the fluorescent material, and the results obtained in the experiments

synaptic contacts There is almost conclusive evidence that these fibres contain NA but it cannot be excluded that they contain also DA or that some of them have DA exclusively The most significant facts are summarized below

1 The fluorescence method shows a high specificity for certain catecholamines and tryptamines (see the introduction to Results) All the characteristics of the reaction and the properties of the fluorescent product indicate that a compound is demonstrated which belongs to one or the other of these groups The colour of the emitted light suggests a catecholamine rather than a tryptamine If the fluorescence is due to a catecholamine the characteristics of the reaction strongly implicate a primary amine and almost exclude a secondary amine such as A The fact that A exists in only very low concentrations in the hypothalamus (BERTLER and ROSENGREN 1959) strengthens the view that this amine can be excluded — The fluorescence method is discussed further in another paper (FALCK 1962)

2 Of the known monoamines which yield intensely fluorescent condensation products with formaldehyde only A NA DA and 5 HT have been found in significant amounts in the hypothalamus of normal animals The corresponding amino acid precursors may develop fluorescence but are normally present — if at all — in too small amounts to interfere

3 After administration of reserpine the disappearance and recovery of the fluorescence as function of dose and time agree very well with those of the catecholamines (CARLSSON *et al* 1957 BRODIE 1958 BRODIE MAICKEL and WESTERMAN 1961 and unpublished data)

4 When the hypothalamic catecholamines — but not 5 HT — are depleted through the administration of m tyrosine and *a* methyl m tyrosine the fluorescence also disappears This finding in itself almost conclusively proves that a catecholamine and not 5 HT is accumulated in the varicose fibres Since *a* methyl m tyrosine under the conditions employed affects DA but slightly the data indicate that the catecholamine in question is predominantly NA

5 *When a reaccumulation of 5 HT — but not* catecholamines — is brought about through administration of nialamide to reserpinized animals no fluorescence reappears in *the fibres*

6 Finally the distribution of NA — but not 5 HT and DA — in the different parts of the hypothalamus (*BERTLER 1961) is similar to* that of the fluorescent varicose fibres which furthermore have been found to be scarce in all brain parts with a low NA content *(e g* cerebellum and cerebral cortex) Table 1 gives the content of this amine in some parts of the rat brain It is seen that the preoptic region which has *abundant varicose fibres* also shows a high concentration of NA The somewhat unexpectedly high content in the posterior hypothalamus may be due to the presence of NA in the big fluorescent nerve cells (see below)

von EULER and his co workers *(cf von EULER 1956 1961)* have presented strong evidence for the view that the adrenergic transmitter is NA and that

SUMMARY

The cellular localization of brain monoamines has been studied with the use of a fluorescence method for histochemical demonstration of certain catecholamines and tryptamines in combination with a pharmacological approach. Mainly the hypothalamus and caudate nucleus of mouse and rat were examined The following are the most important results

1 Noradrenaline in the hypothalamus shows a high accumulation in fine varicose fibres which are present almost everywhere, but highly concentrated to some areas (especially a large area in the preoptic region, the supraoptic and paraventricular nuclei, and the periventricular nuclei in the anterior hypothalamus) There is good evidence that these fibres represent the terminal parts of axons forming synaptic contacts Consequently, adrenergic neurons may exist and noradrenaline may serve as a synaptic transmitter in the central nervous system

2 Similar fibres are present also in other parts of the central nervous system, including the spinal cord More dense accumulations have so far been found only in some areas in the pons and medulla oblongata.

3 There is good evidence that the perikarya and processes of some nerve cells in the hypothalamus (and also other brain regions) contain a catecholamine (probably noradrenaline) in low concentrations These nerve cells may represent the cell b[illegible]

4 [illegible]

centr[illegible]

tion [illegible] called Dopamine may be the main amine present The intimate relation of this zone to the hypophysial portal system suggest a rôle in the humoral regulation of the pituitary functions

5 Some circumscribed and more or less continuous areas, including the amygdala, surrounding the anterior hypothalamus and preoptic region develop a fluorescence which seems to be due the presence of a catecholam[illegible]

[illegible]

6 The caudate nucleus as a whole develops a fairly high fluorescence. All histochemical as well as pharmacological data strongly indicate thas this fluorescence — mainly at least — is due to the dopamine present The amine is probably localized to submicroscopic structures belonging, for instance, to the neuropil

with drugs strongly support this view The cellular localization is unknown but it does not seem probable that a monoamine is present diffusely throughout the tissue A more attractive hypothesis is that the amine is stored in the terminal parts of submicroscopic nerve fibres which lie fairly close together

The caudate nucleus has a very high DA, much lower 5 HT and very low NA content (BERTLER and ROSENGREN 1959, BERTLER 1961) This nucleus as a whole has a fairly high fluorescence which is quite diffuse All data hitherto obtained — histochemical as well as pharmacological — strongly indicate that this fluorescence — mainly at least — is due to the DA present The pharmacological data are summarized in Table 2 The fact that m tyrosine did not cause the fluorescence to disappear completely may probably be explained on the basis that both m tyrosine and its decarboxylation product m tyramine, 2 hours after the last injection are present in concentrations high enough to *produce a significant fluorescence on formaldehyde condensation*

It is quite improble that the DA present in the caudate nucleus has a diffuse distribution in the tissue and it does not seem very likely that it is accumulated in the glia cells throughout the nucleus The amine is thus probably localized to submicroscopic structures belonging for instance to the neuropil

This work has been supported by grants from the Swedish Medical Research Council the Directorate of Life Sciences AFOSR Office of Aerospace Research United States Air Force monitored by the European Office of Aerospace Research under Grant No AF EOAR 61 64 and by a grant (B 2854) from the United States Public Health Service

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OBSERVATIONS ON THE POSSIBILITIES OF THE CELLULAR LOCALIZATION OF MONOAMINES BY A FLUORESCENCE METHOD

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CONTENTS

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Many problems in the field of adrenergic and tryptaminergic mechanisms depend for their treatment on both a direct demonstration of monoamines and an accurate localization of them at the cellular level Several ways have therefore been tried in these laboratories to obtain sensitive histochemical methods for *i a* catecholamines (CARLSSON *et al* 1961 FALCK *et al* 1962, FALCK and TORP 1962) *Perhaps the most promising method is based on the* principle that the amines can be transformed into intensely fluorescent iso quinoline derivatives by condensation with formaldehyde By means of this method it has recently been possible to directly demonstrate the transmitter, NA * in adrenergic nerves (FALCK and TORP 1962 b) and the localization of this amine in the hypothalamus (CARLSSON *et al* 1962, CARLSSON, FALCK and HILLARP 1962)

In the present paper are presented some observations on the specificity and sensitivity of the fluorescence method and its applicability to tissues

* Abreviations used A=adrenaline NA=noradrenaline DA=*dopamine* and 5 HT= 5-hydroxytryptamine

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MATERIALS AND METHODS

The animals (mouse, rat, guinea pig) were sacrificed by decapitation during light ether anesthesia or by an intravenous injection of air (rabbit) Albino rats (200—250 g body wt) were used in all the experimental series Control and experimental animals were in all instances sacrificed at the same time and their tissues treated identically Various tissues from about 800 animals have been examined

Pieces of the tissues to be studied were immediately excised and frozen in propane cooled by liquid nitrogen They were dried *in vacuo* at —35° for 8—10 days After thorough drying the pieces were treated with formaldehyde gas at +80° for 1 hour in a closed glass vessel containing paraformaldehyde. During this treatment the catecholamines and 5-HT condense with formaldehyde to intensely fluorescent products which are not extracted with hot paraffin or xylene The preparations were then infiltrated *in vacuo* with paraffin at +60° for 10 min Sections (8—10 μ) were placed on non fluorescent slides which were warmed just to the melting point of the paraffin They were mounted in Entellan (Merck) or liquid paraffin (non fluorescent cover glasses) and again warmed to melt the paraffin For studies of cytological details the sections were deparaffinized by the careful addition of xylene to the glasses

The fluorescence of the condensation products is relatively well preserved for 2 to 3 days in sections mounted in Entellan but then the fluorescence intensity often rapidly decreases

It was found that the amines in thin tissue sheets (*e g* rat iris) could be demonstrated without freeze drying The tissue was stretched on a slide and allowed to dry in the air at room temperature for 5 to 20 min It was then directly heated with formaldehyde gas washed in xylene and mounted as a whole Somewhat thicker tissue sheets (*e g* mesenterium containing fat) were dried *in vacuo* for 1 hour at room temperature Despite the crudity of this drying procedure, the NA in the adrenergic nerve terminals in the iris can usually be excellently demonstrated without any signs of diffusion — In the early experiments with this technique failures were often encountered It was found however that an excellent or experimentally satisfactory demonstration of the adrenergic terminals — at least in the rat iris — could consistently be obtained if the tissues were excised 10 to 15 minutes after sacrificing the animals No explanation of this surprising finding has been discovered

The formation of intensely fluorescent products from the amines on condensation with formaldehyde is considerably retarded if the formaldehyde gas

is too dry The paraformaldehyde used (Merck) has a satisfactory water content but when it has been used at +80° once or twice the water content may become too low, especially if the reaction vessels are not efficiently sealed This source of failure may easily be overlooked To prevent the paraformaldehyde becoming too dry, after using once it is transferred to an open vessel and kept for at least a day (longer if the laboratory air is dry) in contact with the air at room temperature to allow resorption of water Another precaution, essential when tissues from normal and experimental animals are compared, is to include some pieces of normal tissue in every reaction vessel as a control that the condensation proceeds satisfactorily

If not too large or too many tissue pieces are frozen and dried in the same apparatus, technical failures in these steps are rarely encountered However, it is strongly indicated that tissue pieces of the same size and shape from control and experimental animals should be treated identically and dried in the same apparatus

If freeze drying of the tissues is satisfactory and proper condensation conditions are used, the adrenergic nerve "terminals" practically consistently show a high fluorescence everywhere throughout the tissue pieces It may be of value however, to point out some important precautions and sources of failure

It can be stated as a general rule that the tissue pieces must be handled as carefully as when the freeze drying method is used for cytological studies A good measure of experience in freeze drying technique is essential The temperature of the pieces must not be allowed to rise above −35° before or during the freeze-drying When the drying is completed (with a good safety margin) the pieces are brought to room temperature *in vacuo* before being taken out Precautions must be taken to prevent their exposure for any longer time than necessary to air with a high degree of humidity (If necessary the pieces may be kept *in vacuo* at room temperature for some days) As soon as possible they are treated with formaldehyde gas and then immediately embedded in paraffin After that they may be stored at room temperature for at least some weeks

A fluorescence microscope was used with non fluorescent objectives and a Kodak Wratten filter number 15 or a Schott OG 4 (1 mm) in the tube These filters have a high absorption of light below 510 and 490 mμ respectively The exciting light was provided by an Osram HBO 200 high pressure mercury lamp The light was filtered through a 3 mm or 5 mm Schott BG 12 filter Usually dark field condensors (Zeiss Dunkelfelt- and Ultrakondensor) were used for examination and photography of the sections The condensors used are non fluorescent, have a high resolution power and permit a high intensity of the activating light without the necessity of strongly absorbing secondary filters. For examinations of the activating and fluorescent light an Osram XBO 2001 high pressure xenone lamp was used together with a monochromator (see FALCK et al 1962)

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terns have time to occur The diffusion distance — although short — is sufficient to prevent the detection of NA in adrenergic neurons already when the formaldehyde gas is saturated with water at +50° (For obvious reasons such a diffusion is of no great importance in the adrenal medulla and other aggregates of chromaffin cells) The high lability of the catecholamines in this respect is also demonstrated by the finding that freeze dried sections made in a cryostat at —20° cannot be used for demonstration of NA in adrenergic terminals

Convincing evidence exists that the fluorescence method specifically demonstrates NA in the adrenergic nerve terminals' (Section IV) It was therefore of considerable interest to examine whether the characteristics of the condensation reaction and the properties of the condensation products are the same when NA is present in the tissue stores as in models (NA enclosed in a protein film) In all of the previous experiments (FALCK and HILLARP, to be published) both models and adrenergic nerves were found to behave quite similarly in respect to *i a*

1 The conditions for the development of fluorescence (time, temperature, and their relation to the water content of the formaldehyde gas)

2 The wavelength for maximal activation and the colour of fluorescence

3 The UV lability of the fluorescent product.

4 The binding of the fluorescent product to the proteins and the stability of this binding

5 The disappearance of the fluorescence in water and its reappearance after drying

6 Behaviour of the fluorescent products towards oxidizing reducing and other agents

There is good evidence that DA, A and 5 HT also behave essentially in the same way in models as in tissue stores (FALCK and N.-Å HILLARP, unpublished experiments)

The characteristics of the condensation reaction and the properties of the fluorescent product may consequently be used as further criteria for the specificity of the fluorescence reaction. When these criteria, adequate controls and an experimental pharmacological approach are used in studies of the cellular localization of catecholamines and tryptamines, good possibilities exist for identifying at least some of the amines with a high degree of certainty

Another important point is that the main features in the reaction between the monoamines and formaldehyde and the chemical requirements and mol cular struct [illegible]
are fairly [illegible]
1962) Th [illegible]
chemical [illegible] used with much more confidence than otherwise would be possible

RESULTS AND DISCUSSION

I Specificity of the fluorescence method

The treatment of freeze dried tissues with formaldehyde gas under the specified conditions produces the formation of compounds with intensive green to yellow fluorescence This fluorescence reaction seems to have a high specificity for certain catecholamines and tryptamines It has been examined extensively with the use of model systems (FALCK *et al* 1962 FALCK and HILLARP 1962) and of many different tissues both normal and tissues where the amine content had been experimentally altered (CARLSSON *et al* 1962, CARLSSON, FALCK and HILLARP 1962 FALCK and TORP 1962 b, the present paper and unpublished results) The evidence so far obtained seems to show that other biogenic compounds under the conditions used either cannot develop a fluorescence in the green to yellow range sufficiently strong to interfere with the localization of tissue monoamines or — if so — exist in too small concentrations in normal tissues to be significant in this respect Several compounds normally occurring in the body may be condensed with formaldehyde to fluorescent products but so far all of them have been found to belong to the first category (*e g* histamine) or the second (*e g* 3 4 dihydroxyphenylalanine 5 hydroxytryptophan) Some proteins rich in *i a* tyrosine or tryptophan or both may develop a weak green to yellow fluorescence, which in special cases may produce a disturbing background (the intensity may become higher after prolonged exposure to formaldehyde at +80°) This fluorescence — like the autofluorescence in some tissues — is usually easily distinguishable from that of the monoamines since it is often possible to deplete and to replete the amine stores and also to increase their content in various ways by means of drugs Such a pharmacological approach has been used in this and other studies at these laboratories

Other ways to test whether the observed fluorescence is due to autofluorescent material or to proteins and other compounds that in contrast to the catecholamines do not easily diffuse is to examine the fluorescence in tissues treated at +80° without formaldehyde gas or with the gas saturated with water In the former case only autofluorescent material will be seen A slight possibility exists however that the fluorescence of such material may show up first after formaldehyde treatment namely where the compound is soluble in paraffin (or he mounting medium) before but not after this treatment Under the latt condition — as shown in experiments with varied water contents in the reaction vessels — the catecholamines easily diffuse a short distance before the condensation with formaldehyde and binding to the pro

Fig 1—6 Fluorescence photomicrographs of stretch preparations from the dilator muscle of the rat iris The preparations were dried at room temperature for 5—20 minutes treated with formaldehyde gas from paraformaldehyde at +80° for 1 hour As well as the fluorescent nerve fibres in the muscle, a vascular plexus is clearly visible in fig 1 For further explanations see text

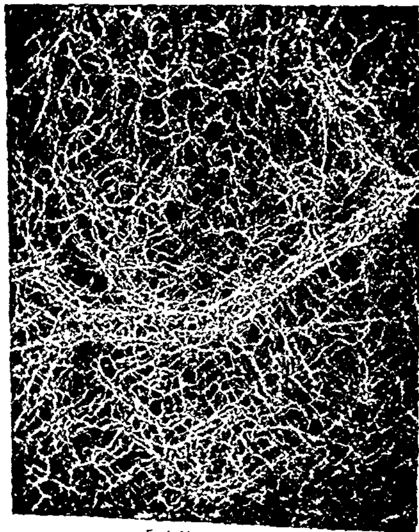


Fig 1 Magnification 150 x

Greater difficulties are encountered in the differentiation between DA and NA on the one hand and 5-HT on the other All three amines react easily and give rise to intensely fluorescent products with fairly similar properties However, it was found in model experiments (FALCK and HILLARP, un-

II *Sensitivity of the fluorescence method*

Experiments with model systems have shown that the products formed from certain catecholamines and tryptamines when condensed with formaldehyde in a dry protein film have a very intense fluorescence (amounts as low as 0.001 μg in a one μl spot of serumalbumin can be detected). Although it is difficult to decide the limit of detection of the tissue amines the results obtained clearly show that the present method (and also another fluorescence method CARLSSON *et al.* 1961) has a sensitivity of another order of magnitude than hitherto available histochemical methods. This is best illustrated by the following findings:

1. The fluorescent product of NA in adrenergic neurons shows a brilliant fluorescence in the varicosities of the terminals with a diameter above 0.3 μ and a weak but distinct *fluorescence in the cell bodies and larger processes.* As judged from data on the NA content of the cranial cervical ganglion (*MUSCHOLL and VOGT 1958*) *the concentration of NA in the cell bodies* is probably less than 100 μg/g.

2. The 5-HT in normal rat mast cells which has hitherto been beyond the reach of histochemical detection gives a very strong fluorescence and single granules from disrupted cells show a high fluorescence (unpublished data).

3. The 5-HT in blood platelets shows a distinct fluorescence when the content exceeds about *100* μg/ml (unpublished data).

III *Possibilities of differentiating between different amines*

No differences have been found in the characteristics of the condensation reaction and the properties of the fluorescent products from DA and NA. The differentiation between these amines must therefore be based on other criteria, *e.g.* their content in the tissues. Fortunately in most tissues one or the other dominates quantitatively.

Catecholamines which are secondary amines (*i.a.* A) can however be differentiated from the primary amines on the basis of their reaction rate. The primary amines are readily condensed with formaldehyde (from paraformaldehyde) also at +50°. Under these conditions the secondary amines do not develop any significant fluorescence. However *if the reaction is performed at* +80° (or higher) for a prolonged time (2—3 hours) A in both models and tissues *(frog heart ventricle adrenal medulla) is transformed to* a highly *fluorescent* product. — No fluorescence is obtained with tertiary amines since ring closure cannot take place.

Under some experimental conditions large amounts of 3-O-methylated catecholamines may accumulate in certain tissues. However the fluorescence developed from these amines at *+80° for 1 hour is too low* to be detected.

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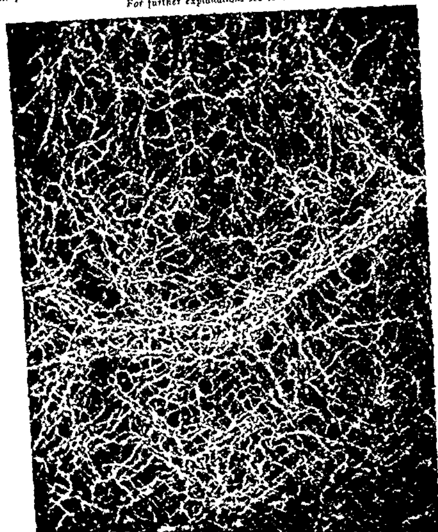


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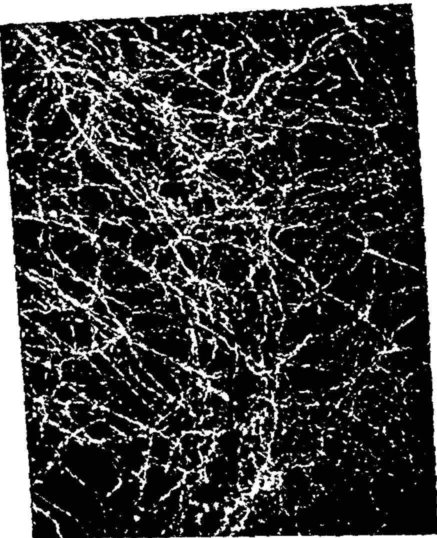


Fig 3 Magnification 375 ×

respectively Examinations of several different tissue structures containing either 5 HT or catecholamines showed that usually this difference is also found in tissues However differentiations between these two categories of amines on the basis of the fluorescence colour must be made with the greatest caution If the content of 5 HT is low or the condensation reaction is not satisfactory (i e too short time or low temperature) the fluorescence may be green yellow or even yellow green If however, the fluorescence developed under proper conditions (and even after prolonged reaction time) is con-

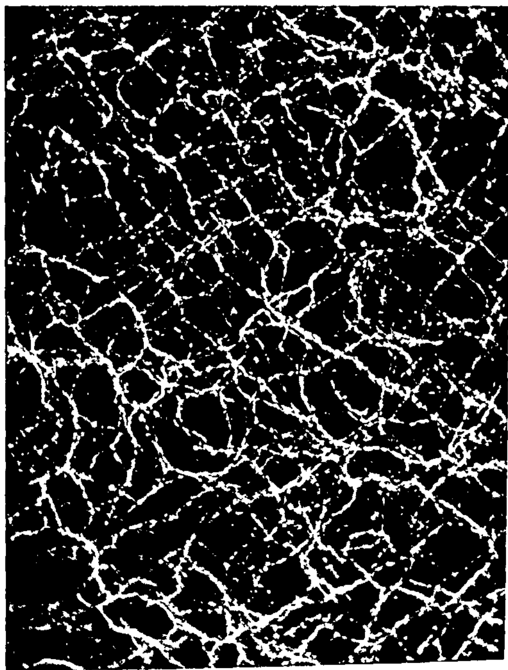


Fig 2 Magnification 375 ×

published experiments) that the fluorescence spectrum obtained on condensation of 5-HT with formaldehyde at +80° for 1 hour (or preferably 2 hours) was distinctly displaced to higher wavelength as compared with that obtained from the catecholamines The difference is clearly observable in the fluorescence microscope the emitted light is yellow and green to yellow-green,



Fig 5 Magnification 600 ×

that this amine is stored in high concentrations only in the "terminals" (von Euler 1956 1961) This accumulated NA could recently be directly demon strated by the use of the present fluorescence method (Falck and Torp 1962 b)

The adrenergic neurons and especially their *terminals* have been studied both in freeze dried tissues and in thin tissue sheets *mounted as a whole* (iris and mesenterium rat) After some experience it is possible to get ex ellen [illegible]

[illegible] as a rich innervation apparatus consisting of a typical autonomic groundplexus (Fig 1—6) (cf Hillarp 1946 1959) Usually one to four very fine axons *run in the anastomosing strands* of the plexus (Fig 4 and 5) The Schwann cell cytoplasm enclosing the axons shows no fluorescence and therefore cannot be seen unless slight diffusion of NA from the axons has taken place (Fig 6) In thicker tissue sheets mounted as a whole there easily occurs a diffusion so marked that the Schwann cytoplasm develops a high fluorescence completely obscuring the axons (Fig 10) The

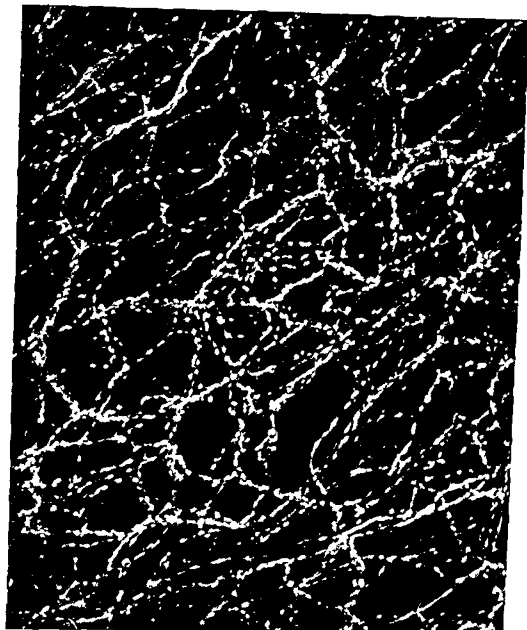


Fig 4 Magnification 600 x

sistently either green to yellow-green or yellow, this so far seems to indicate the presence of, in the first case a catecholamine and in the second a tryptamine

IV. *Demonstration of NA in adrenergic neurons*

Strong evidence supports the view that the adrenergic transmitter, NA, is present in the whole adrenergic neuron of the autonomic nervous system but

Fig 7—10 Fluorescence photomicrographs of stretch preparations of the rat mesenterium These preparations were dried in vacuo and then treated in the same manner as the iris tissue in fig 1—6



Fig 7 Vascular plexus around small arterioles The fluorescent cells are mast cells The fluorescence colour of the latter is yellow, whereas the nerve fibres fluoresce in green Magnification 150 ×

deferens (guinea pig) run everywhere along the muscle cells (Fig 12, 13 and 14) suggesting that every muscle cell comes in *close contact* with one or several axons The abundance of these *preterminal* and *terminal* fibres (Fig 11) may explain the unusually high NA content (about 10 μg/g) of the vas deferens found by Dr N Sjostrand (Dept of Physiology, Karolinska Institutet Stockholm personal communication) However, the construction of the adrenergic innervation apparatus *in this* and other tissues will be reported in another paper

The fibres in the groundplexus are the final ramifications of axons running to the plexus in small nerve bundles (*cf* Hillarp 1959) These latter axons lack varicosities and although thicker *show* a very faint fluorescence which is often seen only when many of *them* are closely bundled together This fluorescence has been examined in the same way as that of the fibres *in the*



Fig 6 Magnification 600 x

round or elongated enlargements of the fibres, the varicosities, which have a thickness of up to 1 μ, show a high fluorescence (Fig 2, 4 and 5) The parts of the axons between the varicosities are very thin and have a fainter fluorescence The finest fibres are just visible with dark-field illumination as a thin, indistinct line between the varicosities and may well be less than 0 2 μ Occasionally a single fibre leaves a strand of the plexus, runs a short distance with or without branching and then seems to terminate

Fig 7—13 may further illustrate the usefulness of the fluorescence method for studies of the adrenergic innervation and its construction in different tissues In many arteries, for example, the innervation consists of a very dense groundplexus (Fig 9—10) which encloses the smooth muscle layer but only in the largest muscle arteries penetrates its superficial zone In contrast to this the adrenergic axons innervating the smooth muscle layers of the vas

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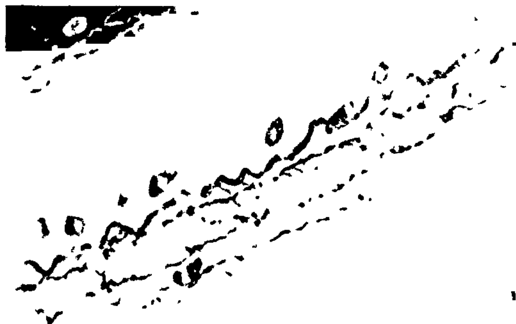


Fig 8 The vascular plexus around a small mesenterial arteriol As well as the nerve fibres in the plexus and the mast cells a fluorecent larger nerve bundle running alongside the vessel is also seen Magnification 375 ×

groundplexus and there is little doubt that it is due to the presence of NA The very low intensity of the fluorescence agrees well with the low NA content of the postganglionic adrenergic nerves examined (*cf* VON EULER 1956)

The cranial cervical ganglion and some other ganglia with adrenergic neurons have also been examined A varying number of the ganglion cells develop a weak green to yellow-green fluorescence in the cell body (except the nucleus) and in the larger processes Although the ganglia have not yet been studied as thoroughly as the nerve terminals all data support the view that the fluorescence is due to the presence of NA Since there is good evidence that the cell bodies of the adrenergic neurons also contain this amine (*cf* VON EULER 1956, 1960) it seems reasonable to assume that the fluorescent cell bodies in fact belong to the adrenergic neurons

The results obtained thus strongly support the view of VON EULER *that the adrenergic transmitter is stored in much higher concentrations in the terminal than in the other parts of the adrenergic neurons They further show that there is an abrupt change — not only in morphology but also in NA content — when the axons enter the autonomic groundplexus postulated* (HILLARP *1946, 1959) to be the innervation structure The findings also suggest that most of the NA in these terminal ramifications is accumulated in the varicosities*



Fig. 9. Vascular plexus around small arteries and a vein in the mesenterium. The arteries are surrounded by a very dense groundplexus, whereas the nerve net around the vein has larger meshes. Magnification 150 ×

The results obtained in the experiments briefly described below so strongly support the histochemical and other data (see also FALCK and TORP 1962 b) that there now is little doubt that the fluorescence method demonstrates NA in the adrenergic fibres.

In a large number of rats the fluorescence reaction of the adrenergic nerves in iris and mesenterium (mounted as a whole) and in extracerebral blood vessels (sections from freeze-dried material) has been examined after denervation (only the iris nerves) and administration of reserpine (subcutaneously),

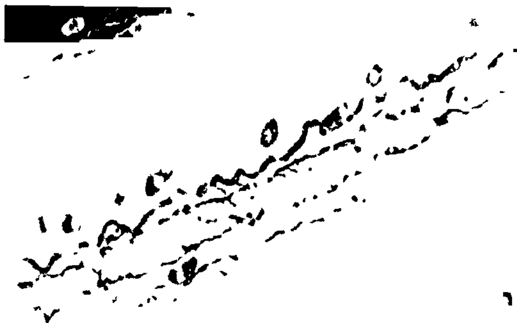


Fig 8 The vascular plexus around a small mesenterial arteriol As well as the nerve fibres in the plexus and the mast cells, a fluorecent larger nerve bundle running along side the vessel is also seen Magnification 375 x

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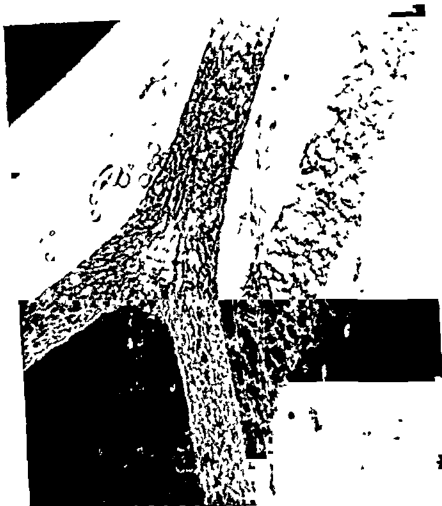


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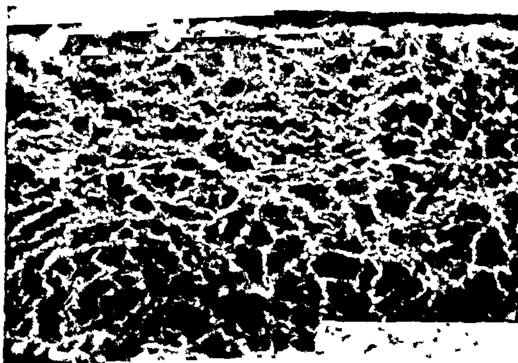
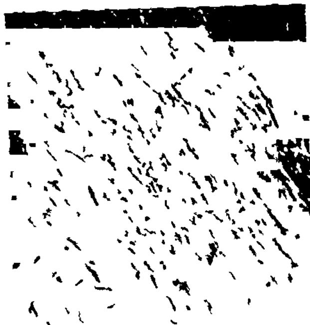


Fig 10 Part of the artery in fig 9 in higher magnification For further explanation see text Magnification 375 ×.

Fig 11—14 10μ transverse sections from vas deferens of the guinea pig The freeze-dried tissue was treated as described above in Materials and Methods



Fig 11 Fluorescent nerve fibres in the longitudinal and circular muscle layers An abundant number of nerve fibres weave through the whole musculature Magnification 100 ×



F g 12 and 13 The c cular muscle layer in higher magn f cations This demonstrates the typ cal morphology of the *term nal axons wi h their n en.ely fluorescent* numerous va cos ties The more d ffuse fluo escent structures are axons out of focus (cf Fg 14) Magn f ca on 250 x and 400 x *resp*



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Fig 12 and 13 The circular muscle layer in higher magnifications This demonstrates the typical morphology of the terminal axons with their intensely fluorescent numerous varicosities The more diffuse fluorescent structures are axons out of focus (cf fig 14) Magnification 250 x and 400 x, resp



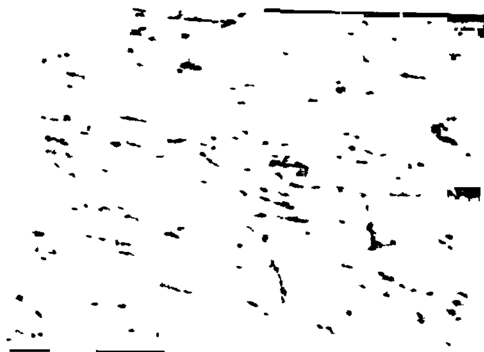


Fig 14 During the exposure the focus was continuously varied The diffuse fluorescent structures seen in fig 12 and 13 then disappear more or less and now show themselves to be varicose axons Magnification 400 x

m tyrosine (intraperitoneally), guanethidine and bretylium tosylate (subcutaneously)

Denervation The fluorescence and morphology of the preterminal and terminal axons were not altered after section of the preganglionic nerves to the cranial cervical ganglion This agrees with the fact that a preganglionic denervation does not influence the organ content of NA (REHN 1958) No definite changes were found up to 20—25 hours after a postganglionic denervation (excision of the cervical sympathetic chain) but several of the animals showed a weaker fluorescence in the denervated than in the normal iris on the other side After 30 hours, however a dramatic reduction of the fluorescence occurred and only some weakly fluorescent varicose fibres could be found These too had disappeared 48 hours after the denervation The findings agree with observations on the disappearance of NA in tissues after a postganglionic denervation (FURCHGOTT 1960 SIDMAN PERKINS and WEINER 1962 WEINER PERKINS and SIDMAN 1962)

Reserpine In dose response experiments (0 15—5 mg/kg body wt time 24 hours) doses of 0 5 mg/kg or higher caused complete disappearance of the fluorescence The lowest dose produced a more or less marked reduction of the fluorescence intensity in the axons which otherwise showed normal appearance When reserpine was injected in the anterior chamber of the eye 1 μg was sufficient to cause the fluorescence to disappear (the injection of

the same amount of solvent in the control eye had no effect) The time course of the disappearance and recovery of the fluorescence was examined after administration of 2—5 mg/kg subcut or 2 μg intraocularly (recovery not examined) Already after 2 hours a more or less marked reduction of the fluorescence intensity could be observed and after 4 to 5 hours no or only very weakly fluorescent fibres were found The first signs of recovery were noted after 3 days A very weak fluorescence in a few axons with typical varicosities had by then appeared One day later most — if not all — of the fibres in the groundplexus and also the nerve bundles fluoresced with a very low intensity No obvious increase in the intensity occurred until 8 days after the injection and recovery was not complete even after 15 days — Thus both the high sensitivity of the fluorescence to reserpine and the time course of its disappearance and reappearance seem to follow those of NA (*cf* CARLSSON *et al* 1957)

Meta tyrosine and a methyl m tyrosine These amino acids — when administered in sufficiently high doses — rapidly deplete the tissue stores of their NA (CARLSSON and LINDQVIST 1962 unpublished data) In agreement with this it was found that the adrenergic fibres in the iris completely or almost completely lost their fluorescence 2 hours after the last of three injections of m tyrosine (800 mg/kg each at intervals of 2 hours) Almost the same marked reduction was found in the adrenergic nerves in the extracerebral blood vessels in animals sacrificed 24 hours after administration of α methyl m tyrosine (400 mg/kg single dose or divided into two with a 3 hours interval)

Guanethidine The animals were examined 24 hours after a single injection of 1—30 mg/kg The intensity of the fluorescence was normal at the 1 mg level but was clearly reduced at the higher dose levels In 4 out of 10 animals given the highest dose the fluorescence had disappeared completely and in the other fibres which otherwise appeared normal developed only a very weak fluorescence However if repeated doses were injected this remaining fluorescence also could be caused to disappear — These findings agree well with observations on the NA depleting effect of guanethidine (GREEN 1962 KUNTZMAN *et al* 1962)

Bretylium tosylate No obvious effect of this drug on the fluorescence or morphology of the adrenergic fibres was observed even when repeated high doses (50—100 mg/kg) were given. Nor has bretylium been found to reduce the NA content of tissues (EULER 1960)

V *The value of the fluorescence method for studies of adrenergic and tryptaminergic mechanisms*

Since the fluorescence method easily demonstrates the presence of adrenergic nerve terminals" the method is obviously of value for studies both of the distribution of the adrenergic innervation to different structures in complex

organs, such as the lung and kidney, and of the construction of the innervation apparatus However, there are many other problems in the field of adrenergic and tryptaminergic mechanisms whose treatment depends on both a direct demonstration and an accurate localization of monoamines at the cellular level Preliminary experiments indicate that the fluorescence method will also be a useful tool for such studies For example, it has been possible to demonstrate (in experiments together with J Haggendal) that in the frog heart ventricle, A is localized to nerve fibres, that in certain mollusc ganglia 5 HT and DA are accumulated in ganglion cells and nerve fibres (DAHL *et al* 1962), that in the rat thyroid gland, cells containing 5 HT are present, and that in some autonomic ganglia in mammals, NA is localized in what appears to be synaptic terminals

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This work was made possible through generous help by Dr A Carlsson and his co-workers at the Dept of Pharmacology University of Goteborg and through grants from the Swedish Medical Research Council AB Leo Halsingborg Sweden the Directorate of Life Sciences AFOSR Office of Aerospace Research United States Air Force, monitored by the European Office Office of Aerospace Research under Grant No AF EOAR 61 44 and through a grant (B 2854) from the United States Public Health Service

Summary

Catecholamines and 5 hydroxytryptamine form highly fluorescent condensation products when treated with formaldehyde

If freeze dried preparations are treated with dry formaldehyde gas (made from paraformaldehyde) at approximately 80° for 1 hour these condensation products are formed in tissues There are no discernable signs of diffusion of the amines from their cellular localizations The methodology has been described and data on the specificity and sensitivity discussed Applications of this method include the demonstration of catecholamines and 5 hydroxytryptamine in neurons and 5 hydroxytryptamine in mast cells (rat and mouse) and blood platelets

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CIRCULATORY RESPONSES TO STIMULATION OF SOMATIC AFFERENTS

with special reference to depressor effects from muscle nerves

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LUND 1952 BERLINGSKA BOKTRYCKERIET

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FROM THE DEPARTMENT OF PHYSIOLOGY UNIVERSITY OF GÖTEBORG
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GÖTEBORG
ELANDERS BOKTRYCKERI AKTIEBOLAG
1962

CHAPTER I

Introduction

a General aspects of cardiovascular control

Modern experimental neurophysiology has clarified to a great extent the anatomical representation and the functional organization of the somatosensory and somatomotor systems in brain and spinal cord. Electrophysiological techniques, especially the methods of single unit analysis, have made it possible to study even some of the more subtle mechanisms engaged in the reflex and central control of the skeletal muscles.

Experimental elucidation of the control of smooth muscles and glands *via* the autonomic nervous system has proceeded far less rapidly. The nervous control of the circulatory system, which is most relevant to the present study, is no exception in this respect. It is true that the peripheral organization of the efferent cardiovascular innervation — the anatomical arrangement, the fibre characteristics and the main functions of the neuro-effectors — has been relatively clearly elucidated (for ref. see Folkow 1955, 1956). There is for instance some direct and indirect evidence on the physiological discharge range of these efferent fibres (*e.g.* Bronk *et al.* 1936, Folkow 1952). The nature, storage, release and decomposition of the chemical transmitter substances in ganglia and nerve endings have been extensively studied (reviewed by von Euler 1960, Hillarp 1960). The specific receptor mechanisms engaged in the control of the cardiovascular system, such as the carotid and aortic baro- and chemoreceptors, are relatively recent discoveries, but some detailed information concerning their afferent impulse discharge is now available and the main principles of their reflex effects are known. Cardiovascular receptors situated in the heart, the central veins and the pulmonary vascular tree are known to exist and their afferent signals have been studied in some detail, but their reflex effects, and hence their exact functional significance, are at present hard to evaluate (for ref. see Aviado and Schmidt 1955, Heymans and Neil 1958). On the other hand little is known about the central nervous integration of the afferent impulses which adjusts the discharge of the efferent visceromotor fibres, and it remains to be further clarified how the tonic activity of some autonomic centres is established and maintained and how their neurone pools are interrelated to produce the efferent impulse messages.

CHAPTER V

CHAPTER VI

reaction The differentiation of the *cardiac* sympathetic innervation into fibre groups which appear to be predominantly inotropic or chronotropic respectively may also be of interest for the present discussion (RANDALL and ROHSE 1956)

By simultaneous recording of blood flows in functionally *different vascular beds* like skin skeletal muscle kidney and intestine during topical stimulation *of cortical* hypothalamic and mesencephalic structures in the cat it has been possible to demonstrate some quantitatively differentiated circulatory responses (LOFVING 1961 a FEIGL JOHANSSON and LOFVING 1962 FEIGL and LOFVING 1962) The efferent discharge patterns initiated by the cardiovascular baro and chemoreceptors have also been studied by means of regional blood flow recordings (LOFVING 1961 a b) These studies give some further indications that sympathetic vasoconstrictor pathways are by no means utilized for generalized and diffuse reactions only It is thus evident that the central nervous arrangement of the vasomotor and cardioregulatory systems provides considerable possibilities for quantitatively differentiated circulatory adjustments

b Scope of present investigation

Recent studies in this department have been concerned with the elucidation of cardiovascular response patterns associated with depressor effects induced from various central structures and peripheral receptors FOLKOW JOHANSSON and ÖBERG (1959) localized a circumscribed area in the anterior hypothalamus from which pronounced blood pressure falls were elicited These could be attributed to a generalized inhibition of tonic sympathetic vasoconstrictor accelerans activity *Such a sympatho inhibitory mechanism* was found to be responsible also for depressor effects obtained by electrical stimulation of an area within the anterior cingulate gyrus (LOFVING 1961 a) The descending fibres from this cortical depressor area appear in fact to pass through the above ment [illegible]

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barorec [illegible] tonic sympathetic activity (LOFVING 1961 a)

Changes in regional blood flow due to a centrally induced inhibition of sympathetic vasoconstrictor fibre activity must be dependent on the actual level of this activity and on the extent of its influence on the effectors of the respective vascular beds Information concerning these matters is thus of the greatest importance for the correct evaluation of neurogenic mechanisms which are responsible for vasodilator responses brought about by inhibition of tonic vasoconstrictor fibre activity There is obviously a multitude of

The efferent discharge patterns in cardio regulatory and vasomotor nerve fibres will adjust the cardiac output and its regional distribution to suit the current demands of the organism as a whole, sometimes in synergism with sometimes in antagonism to the local regulatory mechanisms The regional vascular effects induced by central nervous structures and by afferent fibre systems have not been sufficiently studied and there is consequently a lack of knowledge of the details of many neurogenic cardiovascular response patterns This may partly be due to the old concept of the sympathetic nervous system as being organized mainly for diffuse undifferentiated mass discharge In many studies devoted to the nervous control of the circulation evidence of increased or decreased sympathetic activity has been obtained from observations of some single parameter like mean arterial blood pressure or heart rate These investigations are of course of paramount importance since they have established the general principles of cardiovascular control and they have greatly contributed to the present knowledge of autonomic representation in the central nervous system They give however little or no information concerning the details of the circulatory adjustments Therefore the possibilities of regionally differentiated vascular reactions initiated via the vasomotor fibres should be seriously considered and subjected to experimental investigation (e g FOLKOW 1960 a) In fact some specific circulatory responses induced by more or less selective changes in sympathetic vasomotor and cardioregulatory fibre activity are known and will be briefly referred to below

Hypothalamic thermoregulatory structures exert their direct nervous control of the cutaneous circulation exclusively via the sympathetic vaso constrictor fibres to this region although there is also an indirect dilator mechanism constituted by sweat gland liberation of bradykinin (FOX and HILTON 1958) Selective adjustments of the discharge rate in the vasoconstrictor fibres to the skin are essential for the temperature regulation of man and homeothermic animals (see HERTZMANN 1959 BARCROFT 1960 STROM 1960)

The sympathetic cholinergic vasodilator fibres distributed to the vessels of the skeletal muscles and possibly also to the coronaries form a specific system for nervous control of the blood flow through these vascular regions There is moreover evidence of a remarkably specific distribution of these dilator fibres within the vasculature of the skeletal muscles (FOLKOW MELLANDER and ÖBERG 1961 RENKIN and ROSELL 1962) Their functional significance and anatomical representation within the central nervous system have been the subjects of many studies during the last decade (for ref see UVNÄS 1960 a) According to ABRAHAMS HILTON and ZBROZYNA (1960 a b) this muscle vasodilatation is an autonomic component of the so called defence

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Concerning this older literature the reader is referred to McDowall 1956 pp 32—35 and 70—73) Some of these observations were thus made even before the specific cardiovascular depressor afferents had been demonstrated by Cyon and Ludwig (1866)

Hunt (1895) observed that depressor responses were obtained when weak stimuli were delivered to the central end of a mixed nerve, while pressor effects occurred at higher stimulus strengths He also showed that blood pressure falls were more easily obtained by stimulation of cooled or regenerating nerves On the basis of these findings he suggested that the peripheral nerves contain two types of afferents 'depressor and pressor fibres', characterized by differences in sensitivity to electrical stimuli, cooling etc This interpretation of the results was later criticized by Ranson and Billingsley (1916 d) and Ranson (1921 pp 496—498) who advocated the view that the *opposite circulatory* effects of weak and strong stimuli could be attributed to differences in the organization of the central 'pressor and depressor pathways' (see below) rather than to a differentiation of the peripheral afferent fibres Curiously enough Ranson and Billingsley (1916 a) had previously contributed to the theory of specificity of afferent nerve fibres by demonstrating that an animal s reactions to pain, including the blood pressure rise were abolished by *transection* of the lateral divisions of the dorsal roots where the thinnest afferents are concentrated, but not by transection of the medial divisions which are made up of thicker fibres Hunt s postulate of specific 'pressor and depressor afferents' in the peripheral somatic nerves was however, widely accepted and it gained experimental support in a study by Gordon (1943) The latter investigator showed that the pressor response to electrical stimulation of the sciatic nerve was the first to be abolished if the nerve was subjected to cocainization, which primarily affects the thin nonmedullated fibres If, on the other hand the nerve was subjected to prolonged asphyxia the depressor response was abolished while a rise in blood pressure could still be induced The thicker medullated fibres are known to be more susceptible to asphyxia and it was thus concluded that the depressor responses are evoked by group A afferents while the pressor effects are due to activation of C-fibres The fact that a depressor response to afferent somatic nerve stimulation can be reversed to a pressor effect by *increasing* the impulse frequency without changing the stimulus strength was first demonstrated by Gruber (1917) This was confirmed by Gordon (1943) who concluded that central nervous mechanisms must be able to determine to some extent the direction of the reflex blood pressure change which is consequently not exclusively determined by the type of afferents that are stimulated

It is not until recent years that modern electrophysiological techniques

factors controlling the discharge rate of the vasoconstrictor neurones which constitute 'the final common path' for many central and reflex influences on the vascular smooth muscles For example, possible differences between the neurone pools of the vasomotor centre with respect to their sensitivity to the oxygen and carbon dioxide tension of the blood or to the baro and chemoreceptor fibre activity can be expected to appear as regional differences *in the neurogenic tone of the vessels* (FOLKOW, JOHANSSON and LOFVING 1961) Regional divergences *in these respects were shown to be responsible* for the different degrees of vasodilatation observed in various tissues when the depressor area of the cingulate gyrus the medullary depressor area or the baroreceptors were stimulated (LOFVING 1961 a)

The nature of depressor influences of cortical, hypothalamic medullary and baroreceptor origin has thus been elucidated and the special problems associated with the interpretation of sympatho inhibitory vascular responses have been analyzed in the above mentioned investigations It is well known that depressor — and pressor — responses can be induced also by afferent electrical stimulation of somatic nerves if suitable impulse frequencies and intensities are chosen (see below) *It was considered of importance to perform a more systematic study of the cardiovascular response pattern and the central nervous organization of this 'somatic depressor reflex The present study is mainly concerned with this subject but some observations on the pressor responses which can be obtained by stimulation of somatic afferents will also be included and discussed in relation to other reflex and central pressor responses*

The experimental results will be presented in three different sections

1 Blood pressure responses to stimulation of afferent fibre systems in cutaneous and muscle nerves (Chapter III)

2 Changes of heart rate and of blood flow resistance in functionally different *vascular beds during depressor and pressor responses* to somatic nerve stimulation (Chapter IV)

3 Spinal pathways and bulbar structures engaged in somatic depressor and pressor reflexes *(Chapter V)*

Preliminary results pertaining to the present study have previously been briefly reported (JOHANSSON 1961, 1962)

c *Historical background*

That changes in the arterial blood pressure can be induced by afferent stimulation of various somatic sensory nerves was shown about a hundred years ago (VON BEZOLD 1863 LOVEN 1866 ASP 1867, DITTMAR 1870 etc

Concerning this older literature the reader is referred to McDowall 1956 pp 32–35 and 70–73) Some of these observations were thus made even before the specific cardiovascular depressor afferents had been demonstrated by Cyon and Ludwig (1866)

Hunt (1895) observed that depressor responses were obtained when weak stimuli were delivered to the central end of a mixed nerve, while pressor effects occurred at higher stimulus strengths He also showed that blood pressure falls were more easily obtained by stimulation of cooled or regenerating nerves On the basis of these findings he suggested that the peripheral nerves contain two types of afferents, 'depressor and pressor fibres', characterized by differences in sensitivity to electrical stimuli, cooling etc This interpretation of the results was later criticized by Ranson and Billingsley (1916 d) and Ranson (1921 pp 496–498) who advocated the view that the opposite circulatory effects of weak and strong stimuli could be attributed to differences in the organization of the central pressor and depressor pathways' (see below) rather than to a differentiation of the peripheral afferent fibres Curiously enough, Ranson and Billingsley (1916 a) had *previously contributed to the theory* of specificity of afferent nerve fibres by demonstrating that an *animal s* reactions to pain, including the blood pressure rise, were abolished by transection of the lateral divisions of the dorsal roots where the thinnest afferents are concentrated, but not by transection of the medial divisions which are made up of thicker fibres Hunt's postulate of specific 'pressor and depressor afferents' in the peripheral somatic nerves was, however, *widely* accepted and it gained experimental support in a study by Gordon *(1943)* The latter investigator showed that the pressor response to electrical stimulation of the sciatic nerve was the first to be abolished if the nerve was *subjected* to cocainization, which primarily affects the thin nonmedullated *fibres If*, on the other hand, the nerve was subjected to prolonged asphyxia the depressor response was abolished, while a rise in *blood pressure* could still be induced The thicker medullated fibres are *known* to be more susceptible to asphyxia and it was thus concluded that the depressor responses are evoked by group A afferents while the pressor effects are due to activation of C-fibres The fact that a depressor *response to afferent* somatic nerve stimulation can be reversed to a pressor effect by *increasing* the impulse frequency without changing the stimulus *strength* was *first* demonstrated by Gruber (1917) This was confirmed by Gordon *(1943)* who concluded that central nervous mechanisms must be able to determine to some extent the direction of the reflex blood pressure change, *which* is consequently not exclusively determined by the type of afferents that are *stimulated*

It is not until recent years that modern electrophysiological techniques

have been used to identify more exactly the afferent fibres which are responsible for the various blood pressure changes obtainable by *stimulation of somatic nerves*. LAPORTE and MONTASTRUC (1957) made a systematic study of the effects of *cutaneous* afferents on the arterial blood pressure in curarized decerebrated or urethane anesthetized cats. Slight depressor responses were seen in the decerebrate preparation when the A alpha group was selectively activated and in the anesthetized animals when the A delta fibres were activated at low frequencies. Stimulation of the A delta group at high impulse rates produced pressor reactions. Activation of cutaneous C fibres after electrical blockade of the medullated afferents exerted a pressor influence in both decerebrate and anesthetized animals. A corresponding investigation of the reflex circulatory effects of *muscle* afferents was made by LAPORTE, BESSOU and BOUISSET (1960) on decerebrated or chloralose anesthetized cats. Stimulation of the largest muscle afferents (group I a and b) was shown to have no influence on the blood pressure and an increase of the stimulus strength to activate also group II fibres caused slight pressor responses at high impulse rates in the decerebrate cats. Depressor effects, distinct but rather small (about 10 mm Hg), were seen when group III fibres were activated with low frequencies, especially in the anesthetized animals (see also SKOGLUND 1960). C fibres from muscles, like those from the skin, were shown to exert a pressor influence.

The *central nervous organization of the reflex connections* engaged in the vasomotor responses to somatic nerve stimulation was the subject of a series of publications by RANSON and his co-workers. RANSON and VON HESS (1915) studied the blood pressure responses to afferent stimulation of the sciatic nerves in cats which had been subjected to different spinal cord lesions at the upper lumbar level. They found that the pressor reflex could not be obtained after bilateral transection of the tracts of Lissauer. These tracts, situated around the apices of the dorsal horns, contain the thin afferent fibres which, after entering the cord in the lateral division of the dorsal roots, pass upwards or downwards for a short distance.

The depressor responses, induced by weaker stimulations of the sciatic nerves, could be obtained in the animals with dorsolateral lesions but were abolished by transections of the ventrolateral funiculi of the lumbar cord (RANSON and VON HESS 1915, RANSON and BILLINGSLEY 1916 c). Thus, according to these authors there are in the spinal medulla an ascending pressor path constituted by chains of short neurones within the dorsolateral tracts of Lissauer and another, depressor path, made up of long ascending fibres running within or in close connection with the spinothalamic tracts. The

differences in synaptic resistance between these two pathways were thought to be responsible for the fact that the depressor effects were obtained with stimulus intensities much lower than those required to produce pressor responses (RANSON and BILLINGSLEY 1916 d RANSON 1921 pp 496–498 cf above)

Studies on decerebrate preparations have shown that telencephalic or diencephalic structures are not essential for mediation of the blood pressure responses to stimulation of afferent somatic nerves The organization of the reflex connections in the lower levels of the central nervous system is however incompletely known It has been assumed that the afferent fibres in the spinal nerves exert their main influence on the circulatory system *via* the cardio vascular centres of the medulla oblongata although there is in fact little definite experimental evidence in support of this view Since various somatic and visceral stimuli produce vascular re ponses in chronic spinal animals and in spinal man (BROOKS 1933 SAHS and FULTON 1940 WHITTERIDGE 1960) the existence of neuronal connections between afferent fibre systems and vaso motor neurones within the spinal cord itself is beyond doubt Little is known however about the relative importance of such spinal reflex arcs as compared with afferent ascending connections to the bulbar centres in animals with an intact brain stem

Experimental results indicating a predominance of the hindbrain structures for the mediation of the somatic pressor reflex were presented by RANSON (1916) He showed that bilateral transection at the upper thoracic level of the tracts of Lissauer which had been found to constitute the ascending pressor path (see above) abolished the pressor response to sciatic nerve stimu lation while blood pressure rises could still be evoked by activation of afferent fibres in the brachial plexus entering the cord above the lesions Thus interruption of the ascending pathways for the sciatic afferents above the thoraco lumbar sympathetic outflow eliminated their vasoconstrictor influence while the corresponding effect of forelimb afferents with intact connections to supraspinal centres was well preserved

As far as the mediation of the somatic depressor reflex is concerned attempts have been made by some authors to investigate the role of the so called depressor area of the medulla oblongata It was shown by RANSON and BILLINGSLEY (1916 b) that topical stimulation of a small region just lateral to the obex produced a fall in blood pressure and later experimental explorations of the *medulla* with reference to vasoactive structures have confirmed the existence of a depressor area in this mediocaudal part of the bulb (LIM WANG and YI 1938 WANG and RANSON 1939 a MONNIER 1939 ALEXANDER 1946 BACH 1952 AMOROSO BELL and ROSENBERG 1954) SCOTT

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of circulatory changes elicited by sensory nerve stimulation which did not fit into the scheme of the Loven reflex. The details of these circulatory responses are still insufficiently clarified and their functional implications are poorly understood. In a recent review on nervous cardiovascular control UVNÄS (1960 b, p. 1145) briefly states that satisfactory information is still lacking as to the response patterns associated with depressor and pressor reflexes due to afferent impulses in sensory nerves.

(1925) and LINDGREN and UVNÄS (1954) found that the blood pressure falls obtained by stimulation of vagal afferents were abolished by superficial cauterization of the region of the ala cinerea while the depressor responses to afferent somatic nerve stimulation were not affected by such lesions On the other hand topical application of strychnine nitrate in this region (SCOTT 1925) eliminated also the 'somatic depressor reflex' Further information as to the central nervous arrangement of this reflex is obviously required

The cardiovascular responses associated with stimulation of somatic nerves were rather popular subjects for studies at the beginning of this century but they somehow 'grew out of fashion', probably associated with the new discoveries of baro and chemoreceptor reflexes These latter mechanisms could easily be conceived as important and purposeful factors for the steady control of cardiovascular homeostasis It is interesting to note that the cardiovascular effects of afferent somatic nerve stimulation were almost completely neglected in a recent symposium on the control of the circulation by the central nervous system despite the fact that a separate section was devoted to 'Cardiovascular reflexes initiated from afferent sites other than the cardiovascular system itself (WHITTERIDGE 1960) There are in the older literature some scattered data on the reactions of individual vascular beds induced by stimulation of somatic afferents but the results of different workers were often contradictory and are difficult to evaluate (for a survey of this literature see McDOWALL 1956 pp 32—37 and 75—89) Beside the methodological problems which investigators in cardiovascular research had to overcome in those days their interpretations of the experimental results were necessarily difficult due to lack of basic information concerning hemodynamic principles characteristics of the smooth muscles extent and type of the vasomotor innervation etc For example the concept of reciprocal tonic control of the vascular circuits by way of vasoconstrictor and vasodilator nerve fibres which has later been largely disproved was then widely accepted and the buffering functions of the carotid baroreceptors were unknown until the nineteen twenties

On the basis of the early studies afferent stimulation of somatic nerves was considered to induce both generalized and regional types of cardiovascular reactions (see McDOWALL 1956 pp 32—37 and 75—89) The observations made by LOVÉN (1866) that stimulation of for instance the posterior auricular nerve of the rabbit caused a vasodilatation in the ear together with a blood pressure rise led to the concept of the Lovén reflex This implies that on stimulation of afferents from a certain part of the body a reflex dilatation of the vessels in that particular region accompanied by a vasoconstriction in other vascular beds, will occur There were however, many observations

changes in impulse rate are hemodynamically highly important due to the steepness of the frequency response curves of many vascular beds Simultaneous recordings from *isolated single C fibres* in different tissues would be technically next to impossible and even less significant for the interpretation since one could not be sure that the units recorded from would reflect the mean changes in regional vasomotor fibre discharge which is the decisive factor for the vascular response Further it would be very difficult to ascertain whether the ultimate destinations of the fibres were arterioles precapillary sphincters or veins This might not be without importance since it is by no means impossible that the vasoconstrictor neurones to different consecutive sections of the vascular beds are differently engaged in cardiovascular reflexes (cf FOLKOW 1960 a) These methodological obstacles have recently been discussed by LOFVING 1961 a)

For the purpose of investigating reflex vasomotor adjustments it seems at present more feasible to study experimentally the effector responses in terms of changes in regional vascular resistance and then to evaluate the average shifts in vasoconstrictor fibre discharge rate from the frequency response relationships of different vascular beds as previously reported (CELANDER and FOLKOW 1953 CELANDER 1954) This approach has several advantages The steep frequency response curves for the vascular smooth muscles implies that even small shifts in impulse rate can be disclosed by studying the effector responses Furthermore if regional blood flow is measured the reactions of the smooth muscles of the resistance vessels are greatly magnified in the recordings due to the Poiseuille relationship (the flow being proportional to the fourth power of the radius of the vessels) It must be admitted that such estimations of reflex changes in vasomotor fibre discharge can be somewhat unreliable if the variable influence of non neurogenic factors on the circulatory responses is not kept under control Determinations of frequency response curves should therefore preferentially be made under the prevailing conditions of each separate experiment Approximate calculations of reflex changes in the vasoconstrictor fibre activity based on the above principles and with the above mentioned precautions have been presented by FOLKOW (19 2) and LOFVING (1961 a) Such estimations have been made also in some of the present experiments but the response patterns of the vasomotor reflexes studied [illegible]

vasc [illegible]

Re [illegible]

been [illegible] study by comparing the circulatory response patterns induced from somatic nerves with those elicited in other pressor and depressor reflexes For example if under otherwise identical conditions

CHAPTER II

Methods

a General considerations

The present investigation was undertaken in order to study some of the characteristics of reflex cardiovascular responses which can be elicited by activation of certain afferent fibres in somatic nerves. From the strictly neurophysiological viewpoint such reflexes would seem to be most rationally studied by electrophysiological recordings of efferent impulse discharges evoked in vasomotor fibres. Such an approach might, however, not be entirely satisfactory since, so far as the cardiovascular system is concerned, the important aspects are the responses actually induced at the effector sites, *i. e.* at the heart and blood vessels.

It is true that if certain experimental precautions are taken there is a good correlation between the average constrictor fibre discharge rate and the vascular response in any given circuit (CELANDER and FOLKOW 1953, CELANDER 1954), but the fact that the density of the vasoconstrictor innervation and the effector sensitivity to the adrenergic transmitter vary considerably from one vascular bed to another (FOLKOW 1960 a, b) makes it impossible to predict the resulting hemodynamic responses from direct determinations of discharge rates in regional vasomotor fibres. Moreover, recordings of action potentials from *preganglionic* sympathetic bundles, though relatively easy to perform, would be of limited value for the estimation of regional differences in reflex vasomotor fibre adjustments, since the ultimate vascular destination of the fibres recorded from would only exceptionally be known. This anatomical problem could be at least partially solved by studying instead the electrical activity in bundles of *postganglionic* C fibres isolated in the proximity of the tissues themselves, but difficulties other than the technical ones would still be encountered. The nervous discharge in sympathetic fibres is apt to occur in rhythmic bursts, often synchronous with pulse or respiration (BRONK *et al.* 1936), but these tend to fuse when the discharge rate is increased. A quantitative comparison between the induced changes in the vasoconstrictor fibre activity of different regions would then be a rather intricate task. Very slight differences between various regions would have to be judged since even small

changes in impulse rate are hemodynamically highly important due to the steepness of the frequency response curves of many vascular beds. Simultaneous recordings from *isolated single C fibres* in different tissues would be technically next to impossible and even less significant for the interpretation since one could not be sure that the units recorded from would reflect the mean changes in regional vasomotor fibre discharge, which is the decisive factor for the vascular response. Further, it would be very difficult to ascertain whether the ultimate destinations of the fibres were arterioles, precapillary sphincters or veins. This might not be without importance since it is by no means impossible that the vasoconstrictor neurones to different consecutive sections of the vascular beds are differently engaged in cardiovascular reflexes (cf. FOLKOW 1960 a). These methodological obstacles have recently been discussed by LÖFVING 1961 a).

For the purpose of investigating reflex vasomotor adjustments it seems at present more feasible to study experimentally the effector responses in terms of changes in regional vascular resistance and then to evaluate the average shifts in vasoconstrictor fibre discharge rate from the frequency response relationships of different vascular beds as previously reported (CELANDER and FOLKOW 1953, CELANDER 1954). This approach has several advantages. The steep frequency response curves for the vascular smooth muscles implies that even small shifts in impulse rate can be disclosed by studying the effector responses. Furthermore, if regional blood flow is measured the reactions of the smooth muscles of the resistance vessels are greatly magnified in the recordings due to the Poiseuille relationship (the flow being proportional to the fourth power of the radius of the vessels). It must be admitted that such estimations of reflex changes in vasomotor fibre discharge can be somewhat unreliable if the variable influence of non neurogenic factors on the circulatory responses is not kept under control. Determinations of frequency response curves should therefore preferentially be made under the prevailing conditions of each separate experiment. Approximate calculations of reflex changes in the vasoconstrictor fibre activity based on the above principles and with the above mentioned precautions have been presented by FOLKOW (1952) and LÖFVING (1961 a). Such estimations have been made also in some of the present experiments but the response patterns of the vasomotor reflexes studied in this investigation will generally be described as changes [illegible]

[illegible] this study by comparing the circulatory response patterns induced from somatic nerves with those elicited in other pressor and depressor reflexes. For example, if under otherwise identical conditions

two different kinds of afferent influences initiated blood pressure rises of about the same magnitude but showed differences with regard to the changes of flow resistance induced in various vascular beds this would indicate a dissimilar distribution' of the vasoconstrictor fibre activation in the two cases Such results could also be said to indicate a difference in the convergence of the afferent fibre systems towards differentiated central neurone pools which determine the efferent vasomotor fibre activity to various tissues Experimental studies of circulatory effector responses alone can thus be expected to reveal also some details of the functional organization of central vasomotor reflex connections

Part of the present study has been devoted to the *central nervous arrangements* implicated in the circulatory reflexes initiated from somatic nerves of muscular or cutaneous origin These latter experiments are concerned with the spinal pathways and the central reflex stations involved

To determine the location of spinal pathways essential for the mediation of these reflexes the influence of various acute cord lesions upon the blood pressure responses were studied If a certain vasomotor response to an afferent peripheral stimulus is selectively eliminated by a severance of some spinal funiculus above the level of the thoraco lumbar sympathetic outflow this suggests that the reflex studied is mediated over supraspinal cardiovascular centres and that its ascending pathway has been interrupted by the lesion Such a conclusion is valid however only if it can be shown that the abolition of the response is not simply due to a non specific decrease of cardiovascular reactivity or to a severance of the descending bulbospinal vasomotor fibres Therefore the functional integrity of the bulbar vasomotor centre and its descending connections was regularly controlled by observing the cardio vascular responses to carotid artery occlusion and/or afferent vagal nerve stimulation before and after the lesions had been produced Abolition of a reflex response to an afferent peripheral stimulus by a localized spinal cord lesion is a type of negative finding which should be supported by positive evidence from experiments in which the funiculi supposed to contain the ascending pathways are left intact Despite such precautions there remains the theoretical possibility that the tract referred to does not correspond to an ascending pathway but to a descending conditioning one working for example by facilitating propriospinal reflex connections The latter possibility would probably be supported by the presence of a local sign in the reflex response pattern but would not be disproved by its absence (cf Chapter V) With regard to this question of ascending *versus* descending conditioning tracts it is valuable to compare the results of cord lesions with available information on spinal pathways obtained in studies with other techniques For example electro

physiological recordings of spinal potentials evoked by afferent stimulation and neuro anatomical degeneration studies have mapped out the courses of the chief ascending fibre systems

In the postexperimental control of the size and localization of lesions it must of course be recognized that the functional damage may extend beyond the limits of the anatomical one seen under the microscope

The action of the *anesthetic agent* presents a special problem when reflex mechanisms are studied in animals under anesthesia It is possible, for instance that in the anesthetized animal the synaptic transmittability in the normally predominant reflex pathways may be depressed or conversely, a reflex con nection may be released from a higher central control Anesthetics can be avoided by using decorticate or decerebrate preparations but this exclusion of the higher levels of the central nervous system may also produce 'abnormal reflex conditions as compared with those in an animal with an intact brain On the other hand experiments performed on intact unanesthetized cur arized animals do not offer a solution to the problem either Beside the fact that such experiments are questionable from an ethical point of view, the emotional state of the animal might be so excited that any type of afferent stimulus might produce some non specific more or less vivid defence alarm reaction In the face of these difficulties the most reasonable compromise seems at present to be to study a reflex mechanism both in decorticate or decerebrate preparations and in animals anesthetized with different agents

b Material

118 cats of both sexes with body weights varying between 2 0 and 4 9 kg were used Of this total number of experiments 21 were discarded as unsuccess ful either because the animals died or deteriorated markedly at an early stage or because the results were definitely inconclusive or unreliable due to technical inaccuracies For example the experimental lesions produced in the cervical cord sometimes encroached upon the descending vasomotor fibre system to such an extent that the animals displayed afterwards the reduced arterial blood pressure and circulatory areflexia typical of acute spinal prep arations The experimental results presented are thus based on a number of 97 cats

c Anesthesia and general operative procedures

The anesthetic used in the majority of the experiments (85) was chloralose (Chloralose purissimum Merck dissolved in saline) in a total amount of 25—40 mg kg bodyweight After induction of anesthesia with ether chloralose

two different kinds of afferent influences initiated blood pressure rises of about the same magnitude but showed differences with regard to the changes of flow resistance induced in various vascular beds this would indicate a dissimilar distribution of the vasoconstrictor fibre activation in the two cases *Such results could also be said to indicate a difference in the convergence of the afferent fibre systems towards differentiated central neurone pools which determine the efferent vasomotor fibre activity to various tissues Experimental studies of circulatory* effector responses alone can thus be expected to reveal also some details of the functional organization of central vasomotor reflex connections

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sympathetic control of various effector organs as compared with the direct innervation (CELANDER 1954 MELLANDER 1960) it may affect to some extent the general and regional circulatory responses studied To eliminate reflex changes in the adrenomedullary output the right suprarenal gland was tied off with ligatures and the left one denervated by section of the adrenal branches of the splanchnic nerves on that side This was done in most of the experiments where regional blood flow was recorded The production and output of cortical hormones in the left suprarenal gland will not be disturbed by these procedures

d Recordings of cardiovascular parameters

Mean arterial *blood pressure* was recorded continuously in all experiments by means of a mercury manometer A cannula inserted into one of the common carotid arteries one of the femoral arteries or the inferior mesenteric artery was connected to the manometer by a polyethylene tube filled with saline to which a small amount of heparin was added to avoid coagulation of blood within the cannula

Regional blood flows were measured continuously by means of flow recording units each consisting of an optical drop counter applied to a closed perspex drop chamber and operating an ordinate writer This recording unit is a slight modification of the one described by CLEMENTZ and RYBERG (1949) The venous outflow from the vascular bed to be studied was diverted via a polyethylene tube to the closed drop chamber in which the blood drops fell through inert silicone oil (LINDGREN 1958) The blood was returned to the animal through another polyethylene tube inserted into the proximal end of a suitable vein The flow resistance of this system was reduced to a minimum by using tubes as wide and short as possible In all experiments where blood flow was recorded heparin was given intravenously in doses of 2–5 mg kg body weight (1 mg of the substance used corresponded to 120 I U)

In the recordings obtained with the above method the heights of the ordinates are *inversely* proportional to the volume flow of blood There is a minor divergence from this relationship due to variations in drop size and also to differences in the rising time falling time quotient of the ordinate writer at different rates of flow but these errors tend to cancel each other By means of calibration curves for the separate recording units the ordinate heights can be directly converted to ml min From blood pressure and blood flow recordings regional vascular resistance can then be approximately calculated in terms of peripheral resistance units (PRU) here expressed as mean blood pressure (mm Hg) divided by blood flow (ml min 100 g tissue) This corresponds to the PRU_{100} of GREEN (1948 p 243)

was administered intravenously *via* a cannula inserted into one of the brachial veins. In 5 cats chloralose in doses as above was given in combination with urethane (100 mg/kg). Dial (Ciba) 30 mg/kg intravenously was used in 2 animals. 5 experiments were made on decerebrate cats. The decerebrations, performed with the animals under ether, were made in 2 cats by supracollicular transection of the brain stem *via* a posterior opening in the skull and in 3 cats by the ischemic method (POLLOCK and DAVIS 1923).

In all experiments a *tracheal cannula* was inserted to avoid respiratory complications, facilitate the removal of saliva and mucus from the respiratory tract and to permit artificial ventilation. The *common carotid arteries* were prepared free bilaterally and could be occluded if desired. The *vagal nerves* were carefully dissected at the cervical level so that they could be cut and stimulated in the afferent direction. The *body temperature* of the cats was controlled during the experiments by means of a thermometer in the rectum or in the pharynx and was kept at 37°—38° C with the aid of an electrical heating pad on the operating table. *Dextrane Tyrode* solution was given intravenously to substitute incidental blood losses.

After the completion of the operative preparations for blood pressure and blood flow recordings, nerve stimulations etc. (see below) the animals were *curarized* with gallamine triethiodide (Flaxedil, May & Baker) to avoid disturbances from spontaneous or reflex movements of the skeletal muscles on the vasomotor reactions studied. Flaxedil was given in amounts of 2—4 mg/kg intravenously and, if necessary, in supplementary doses during the course of the experiments. This agent does not have the pronounced effects on the cardiovascular system displayed by tubocurarine and is therefore to be preferred in studies on adrenergic vasomotor control. It has, however, a moderate vagolytic action which may make it less suitable for experiments on cholinergic autonomic mechanisms (see e.g. McINTYRE 1958 p. 128). *Artificial respiration* was administered *via* the tracheal cannula by means of an adjustable respiration pump ("Starling ideal pump", Palmer) which was set at appropriate ventilation volumes. Thus, before Flaxedil was given the pump was geared to a stroke frequency which approximately corresponded to that of the animal's normal breathing and the tidal volume was then so adjusted that it barely suppressed the spontaneous respiration.

Elimination of the adrenal medullae. Reflex changes in the adrenomedullary output of catechol amines have been described in connection with vasomotor reflexes induced from peripheral somatic nerves. As demonstrated by VON EULER and FOLKOW (1953) these changes are not only quantitative but also qualitative since the relative amounts of adrenaline and noradrenaline may be altered. Even if this humoral factor is of subordinate importance for the

sympathetic control of various effector organs as compared with the direct innervation (CELANDER 1954, MELLANDER 1960) it may affect to some extent the general and regional circulatory responses studied. To eliminate reflex changes in the adrenomedullary output the right suprarenal gland was tied off with ligatures and the left one denervated by section of the adrenal branches of the splanchnic nerves on that side. This was done in most of the experiments where regional blood flow was recorded. The production and output of cortical hormones in the left suprarenal gland will not be disturbed by these procedures.

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Venous outflow from *skeletal muscle* was mostly obtained by cannulation of the femoral vein below the inguinal ligament The proximal end of the femoral vein was used for the venous inflow from the recorder To avoid the time consuming and traumatizing procedure of skinning the leg and yet to minimize the admixture of cutaneous blood a tight ligature was tied around the ankle The paws with their richly vascularized pads were thus excluded from the circulation In the remaining part of the hind limb skeletal muscle tissue constituted about 75—80% of the weight and the outflow from the femoral vein was therefore considered sufficiently representative of muscle blood flow It is obviously difficult to determine the exact amount of tissue which is drained by the femoral vein and the numerical values for the vascular resistance of this region will consequently be somewhat unreliable In three control experiments venous outflow from the popliteal vein was recorded after skinning the leg between the knee and the ankle joint in combination with ligation of the paw After the skin had been dissected free it was again wrapped around the calf to protect the musculature from drying and cooling There were no significant differences in the results of these three experiments as compared with those of the main group

Determination of *cutaneous* blood flow was made by cannulation of the great saphenous vein at the ankle level Most of this blood comes from the skin including the pads with their arteriovenous anastomoses while the small paw muscles tendons and bones contribute but little to the saphenal blood The inflow cannula was usually inserted into the proximal part of the saphenous vein on the thigh

In experiments where renal or intestinal blood flow was to be recorded the abdomen was opened in the midline To gain access to one of the *renal* veins it was found most convenient to extirpate the intestine from the duodenum to the rectum In most cases venous outflow was taken from the left kidney mainly because of the frequent occurrence of double veins on the right side The preparation of the vessels was done with the greatest caution to avoid damage of the renal innervation Engorgement of the kidney during the cannulation was prevented by temporary occlusion of the proximal abdominal aorta or of the dissected renal artery The insertion of the cannula required 1—2 min The renal outflow was returned to the animal from the flow recorder *via* one of the jugular veins

The proximal part of the jejunum was chosen for studying the *intestinal* blood flow The rest of the gut was extirpated and the jejunal loop was isolated with its normal vascular and nervous supply intact in the mesenteric pedicle The superior mesenteric vein draining this loop was cannulated while the abdominal aorta was temporarily clamped just under the diaphragm in order

to avoid engorgement of the intestine The blood was returned *via* one of the jugular veins

To be able to compare in the same experiment the degree of reflexly induced changes in the blood flow resistance of different tissues venous outflow was generally measured in two or three vascular regions simultaneously A recording of skeletal muscle blood flow was included in most of these experiments and was usually combined with a recording from one or two other vascular regions kidney intestine or skin The reflex adjustments of the latter vascular beds could thus always be compared with those of the muscle vessels which also facilitated the comparison of data obtained in different animals (cf LÖFVING 1961 a)

In experiments on reflex changes of regional circulation it is often desirable to keep the *arterial inflow pressure* of the vascular beds constant This procedure will eliminate hemodynamic effects on the regional blood flow caused by changes in the pressure head over the region and in the transmural pressure of the vessels so that the recorded shifts in local blood flow will reflect more exactly the neurogenically induced changes in regional vascular resistance The inflow pressure was kept constant by adjusting a clamp applied around the proximal abdominal aorta

The pressor and depressor reactions induced from the peripheral somatic nerves would normally be counterbalanced by the carotid and aortic baro receptor reflexes and might be considerably modified by these cardiovascular buffer mechanisms Therefore in most of the experiments the influence of the baroreceptors was reduced by bilateral cervical vagotomy together with occlusion of the common carotid arteries The latter procedure may sometimes be less effective in reducing the baroreceptor activity due to the existence of collateral connections (CHUNGCHAROEN *et al* 1952) Therefore in some experiments the sinus nerve was cut on one or both sides a procedure which eliminates of course both the baro and chemoreceptor influence from the carotid region The pressor response obtained by carotid occlusion may not be exclusively caused by the reduced baroreceptor inhibition but an activation of the chemoreceptors may also be involved (LANDGREN and NEIL 1951)

To demonstrate the effects of the afferent stimulation on sympathetic vasoconstrictor fibre activity only cholinergic autonomic mechanisms were blocked by the administration of atropine 0 5 mg/kg intravenously in the course of the experiments

Changes of regional blood volume Beside reflex changes in the vasomotor fibre control of the resistance vessels the circulatory responses to stimulation of somatic afferents may include alterations in the neurogenic tone of the so called capacitance vessels which correspond roughly to the venous section

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electrodes and covered with liquid paraffin kept at body temperature Square wave stimuli with variable impulse frequency duration and intensity were delivered by a Grass Stimulator type S4 *via* a stimulus isolation unit

Attempts were made in some experiments to identify the types of muscle afferents which are responsible for the reflex changes in arterial blood pressure obtained by muscle nerve stimulation This was mostly accomplished by comparing in one and the same nerve the strength duration curves for these afferent fibre systems with that of the alpha efferents The following procedure was used Before Flaxedil was given and before the dissected muscle nerve was cut it was stimulated in the efferent direction with stimulus strengths just enough to cause muscle twitches This was performed at different pulse durations and an approximate strength duration curve for the alpha efferents was thus obtained The animal was curarized the nerve was cut distally and the polarity of the electrode was reversed Threshold values for initiating depressor and pressor responses were now determined by afferent stimulation with various pulse durations The strength-duration curves thus obtained for the afferent fibre systems could then be compared with that plotted for the efferent fibres

In one experiment[1]) the activation threshold of the 'depressor afferents' in muscle nerves was determined in relation to that of the group I afferents The stimuli consisted here of condenser discharges half time of decay 45 μsec The blood pressure responses to afferent hamstring nerve stimulation and the action potentials evoked in the ipsilateral dorsal root S 1 were simultaneously recorded A triphasic potential recording was obtained by means of a surface electrode placed at the posterior root The threshold stimulus for the group I afferents was thus determined electrophysiologically and the stimulus strength was then gradually increased until depressor responses were observed in the blood pressure recording The activation threshold of the depressor afferents could thus be related to that of the group I fibres and the former group of afferents identified on the basis of previous neurophysiological studies (ECCLES and LUNDBERG 1959)

Hypothalamic stimulation Electrical stimulation within the area of the anterior hypothalamus from which heat loss mechanisms can be evoked (MAGOUN *et al* 1938 ELIASSON and STRÖM 1950) was done in 5 experiments where cutaneous blood flow was recorded An appropriate part of the parietal bones *on both sides of the midline* was removed with a drill and the head of the cat was mounted in a modified Horsley Clarke apparatus Two concentric electrodes fixed in a moveable holder on the scale frame of the stereotaxic

[1]) I am greatly indebted to Dr ULF NORRSELL for invaluable help with this experiment

of the vascular beds (see FOLKOW *1960 b* MELLANDER *1960)* Adjustments of the tonic activity in the vasoconstrictor fibres distributed to the capacitance vessels will produce changes in the regional blood content A method for investigating simultaneously the responses of the different consecutive vascular sections in a skin muscle region of the cat was described by MELLANDER (1960) This method implies in principle that the reactions of the resistance vessels are studied as changes in regional blood flow while arterial inflow and venous outflow pressures are kept constant and the responses of the capacitance vessels are shown in a plethysmographic recording as characteristic rapid changes in regional tissue volume (*i e* regional blood volume) This method has recently been modified for studying similarly the *reactions of the consecutive vascular sections in the intestine* (FOLKOW LUNDGREN and WALLENTIN 1962) In the present study this latter technique has been used in a few preliminary experiments in order to find out whether reflex reactions of the intestinal capacitance vessels accompany the blood pressure responses to afferent somatic nerve stimulation For details concerning the experimental procedures and the interpretation of the data see MELLANDER (1960) and FOLKOW LUNDGREN and WALLENTIN (1962)

Heart rate was determined in 6 experiments This was done either by counting the pulse rate from the arterial blood pressure recording when driving the kymograph at a relatively high speed or by using a simple pulse recorder which operated an ordinate writer These experiments were designed to demonstrate the possible effects of afferent somatic nerve stimulation on the vagal and sympathetic nervous control of the heart Special attention was paid to the depressor responses induced from muscle afferents and the changes in heart rate accompanying this reflex were studied before and after vagotomy *Flaxedil was not given in these experiments since its slight vagolytic* effect (see above) might conceal reflex changes in the parasympathetic control of the heart

e Stimulation technique

The bundle of nerves supplying the biceps semitendinosus and semimembranosus muscles of the thigh was mostly used for stimulation of muscle afferents This will be referred to below as the hamstring nerve In some cases one of the gastrocnemius nerves or the nerve to the quadriceps muscle was chosen for this purpose Nerve fibres of cutaneous origin were stimulated in the saphenal sural or superficial peroneal nerves Mixed nerves like the median ulnar or sciatic were used exceptionally The nerves were prepared free for a suitable length cut at the distal end placed on bipolar Ag AgCl

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To make selective lesions within the ventral part of the bulbar 'depressor area' the pyramidal surface of the medulla was exposed by a parapharyngeal route as previously described for the cord. A limited area of the base of the skull in front of the foramen magnum was removed with a drill and after opening of the dura lesions could be produced from the ventral aspect of the medulla. These had to be performed with special caution to avoid bleedings from major branches of the vertebral or basilar arteries.

Histological technique. After completion of the experiments with bulbar or spinal cord lesions the respective parts of the nervous system were taken out for histological examination. The material was fixed in 10% formalin, embedded in paraffin and cut in series at 50 μ. These sections were examined unstained with low magnification and the size and localization of the dissections or incisions thus determined. The total extent of the anatomical lesions as seen from the whole series of sections was illustrated in schematic drawings, and photomicrographs were taken from sections of special interest.

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f Operative procedures on spinal cord and medulla oblongata

To study the spinal pathways which mediate the vasomotor effects of afferent somatic nerve fibres the reflex blood pressure responses to peripheral nerve stimulation were recorded before and after various acute lesions within the *cervical spinal cord* When lesions were to be produced within the dorsal half of the cord the posterior neck muscles were divided longitudinally in the midline after which the spinal processes and the arches of the 4th—7th cervical vertebrae were removed with a rongeur The dura was cut open and the dorsal surface of the cord thus exposed Under low magnification the dorsal and/or dorsolateral funiculi were severed with tiny forceps At the beginning of this experimental series the lesions were made with a small knife or a pair of iris scissors but the method of dissections was later preferred since it proved easier to control caused much less bleeding and was more appropriate for the subsequent histological examinations In order to make lesions in the anterior half of the cervical cord the ventral aspect of the atlas was exposed by a parapharyngeal approach The medial part of its ventral arch and the dens of the epistropheus were removed with bone nippers or a drill and the dura was opened from the midline Through this opening it was possible to make lesions within the ventral quadrants of the cord by means of the fine forceps

In order to evaluate the importance of the so called depressor area in the *medulla oblongata* for the mediation of the depressor responses obtained from afferent somatic nerves localized destructions were made within the mediocaudal part of the bulbar reticular formation In order to gain access to the dorsal surface of the medulla the posterior neck muscles were divided and the medial part of the occipital bone was removed with a drill The atlanto occipital membrane and the dura were cut open and the medial posterior part of the cerebellum mainly the vermis was sucked out so that the bottom of the fourth ventricle became visible This could regularly be done without any considerable bleeding Lesions in the caudal portion of the rhomboid fossa and adjacent bulbar regions were mostly produced by dissections with fine forceps In a few preliminary experiments electrolytic lesions were made in these medullary areas

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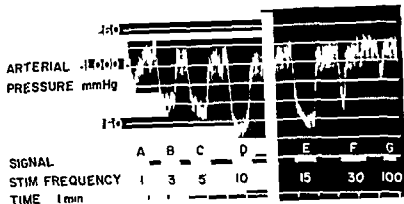


Fig 1 Cat 3 5 kg Chloralose Effects on the arterial blood pressure of afferent stimulation of the left hamstring nerve with 1 5 V and 0 " msec at d fferent impulse frequencies. Pronounced and sustained blood pressure falls are obtained in the low frequency range (3—15 impulses/sec) while higher impulse rates give smaller, transient depressor responses The animal was atropinized, curarized and kept under artificial respiration The vagi were cut in the neck and the common carotid arteries occluded

arteries Under these circumstances stimulation of the hamstring nerve with 10—15 impulses sec produced depressor responses amounting to 60—70 mm Hg while there were smaller transient falls in blood pressure at 30 and 100 impulses sec The depressor effects can be attributed to an inhibition of the tonic activity in sympathetic vasoconstrictor accelerans fibres since vagal inhibition of the heart and 'active cholinergic vasodilatation were prevented by vagotomy and atropinization The correlation between the impulse frequency of the afferent stimulation and the reflex circulatory response illustrated in Fig 1 has been regularly observed in the present series of experiments The depressor effects have thus been most pronounced in the *range of 5—20* impulses/sec while the blood pressure falls diminished and subsided in spite of continued stimulation at impulse rates above 30—40 impulses sec A reversal of the response does not seem to occur at these moderate stimulus intensities (see below) since slight depressor effects were obtained even at impulse rates up to 400 sec in the anesthetized *animals*

The stimulus strength required to produce small but definite blood pressure falls when afferent muscle nerves were stimulated at the optimal frequency of 5 20 impulses/sec was usually in the range *of 0 5—1 5* V, the actual value being of course also dependent upon the pulse duration (see below) At a further stepwise increase of the stimulus strength the depressor responses were enhanced up to a certain point but decreased again at strong stimulus inten

CHAPTER III

Blood pressure responses to stimulation of somatic afferents

The main characteristics of the reflex blood pressure responses elicited from somatic nerves are relatively well known from previous investigations and the afferent fibre systems responsible for these circulatory effects have recently been identified (see Chapter I)

The blood pressure changes observed in the present experiments when afferent spinal nerves were stimulated showed some remarkable quantitative divergencies as compared with those reported in recent studies This was considered to necessitate a re investigation of the general circulatory effects before the details of the cardiovascular response patterns and the central reflex organization could be adequately examined The present chapter will be devoted to a description of the blood pressure effects observed and further to an analysis of certain factors which could be expected to cause the above mentioned quantitative differences Attempts have thus been made to identify the afferent fibre groups which were responsible for the reflex circulatory changes under the present experimental conditions The influence of the anesthesia will be discussed in connection with the results obtained in unanesthetized decerebrated animals and other variations in the experimental conditions such as changes in the functional state of the baroreceptors will also be considered

Results

a Electrical stimulation of muscle nerves

Afferent electrical stimulation of muscle nerves like the hamstring or gastrocnemius nerves with low impulse frequencies and moderate intensities was regularly seen to induce blood pressure falls often of a considerable magnitude This is shown in Fig 1 taken from an experiment where the left hamstring nerve was stimulated with different impulse frequencies varying between 1 and 100 impulses/sec while voltage and pulse duration were kept unchanged (1[illegible] V and 0 7 msec respectively) The high prestimulatory blood pressure of the animal was due to an elimination of the baroreceptor influence produced by bilateral cervical vagotomy and occlusion of the common carotid

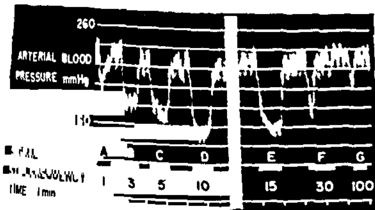


Fig 1 Cat 2 3 kg Chloralose Effects on the arterial blood pressure of afferent stimulation of the left hamstring nerve with 1 5 V and 0 7 msec at different impulse frequencies Pronounced and sustained blood pressure falls are obtained in the low frequency range (3 to impulses sec) while higher impulse rates give smaller transient depressor responses. The animal was atropinized curarized and kept under artificial respiration The vagi were cut in the neck and the common carotid arteries occluded

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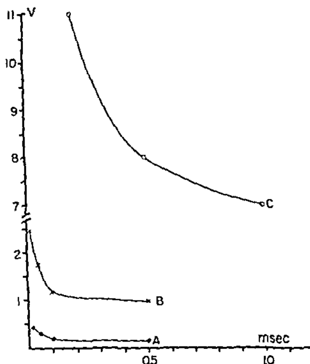


Fig 3 Strength duration curves obtained for the alpha-efferents (A), the depressor afferents (B) and the pressor afferents (C) of the hamstring nerve in a chloralose anesthetized cat The activation threshold of the depressor afferents is about 7 times that of the efferent fibres and the stimulus strengths required to produce pressor effects is about 7 – 8 times higher still (The ordinate scale is discontinuous)

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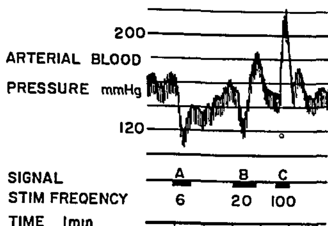


Fig 2 Cat 2 7 kg Chloralose Effects on the arterial blood pressure of strong afferent stimulation of the hamstring nerve (12 V 0 5 msec) at different impulse rates A depressor response is obtained with low frequency stimulation (A) while a pressor effect is evoked at the high impulse rate (C) An intermediate frequency (B) gives a biphasic response The animal was atropinized curarized and kept under artificial respiration The vagi were cut in the neck and one of the common carotid arteries was occluded

sities (8—10 V or more) If higher frequencies were used at such high voltages abrupt pressor reactions were obtained Fig 2 shows this latter phenomenon in an experiment where the hamstring nerve was stimulated with 12 V 0 5 msec and different impulse frequencies At 6 impulses/sec the depressor effect predominated at 20 impulses/sec there was a biphasic response and at a rate of 100/sec a clear cut blood pressure rise was obtained

In accordance with previous studies these findings indicate that the muscle nerves contain at least two types of afferent fibres which influence cardio vascular nerve centres One fibre group which induces depressor responses has a relatively low activation threshold and a low frequency optimum Another group of fibres with a high activation threshold is responsible for the pressor effects The general pattern of response described above seemed to be the same irrespective of which muscle nerve was stimulated Quantitative differences with regard to the magnitude of the blood pressure responses obtained from different muscle nerves seemed to be related simply to the size of the nerve stimulated and hence to the number of fibres activated Thus for example the maximal depressor effects induced from the hamstring nerves were regularly larger than those elicited from the thinner gastrocnemius nerves

A separate series of 8 experiments was performed in order to find out which types of muscle afferents were responsible for the marked depressor responses

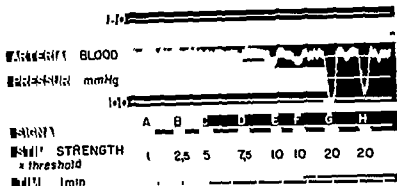


Fig 4 Cat 2 5 kg Chloralose Effects on the arterial blood pressure obtained by graded afferent stimulation of the hamstring nerve with 9 impulses sec The stimulus strengths are given as multiples of the activation threshold for the group I afferents determined by means of a triphasic recording of action potentials from the intact ipsilateral dorsal root S 1 Note that the depressor responses occur at intensities greater than 10 times threshold for the group I fibres

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...ing and recording systems are of importance in this respect

b *Electrical stimulation of cutaneous nerves*

As to the influence of afferent fibres in cutaneous nerves upon the cardio vascular system the blood pressure effects of graded stimulation of the sural saphenal and superficial peroneal nerves were studied in a series of 10 experiments A representative illustration of the blood pressure changes obtained from a ...

... strength was increased in several steps while the pulse duration was kept constant at 0 2 msec At 0 5 V no definite blood pressure response occurred at the low frequency stimulation but a slight depressor effect was obtained with 60 impulses sec (A and B respectively)

sponding values for the high threshold muscle afferents responsible for the pressor effects the same nerve was stimulated with 70 impulses/sec At this impulse frequency the depressor responses were negligible and did not significantly disturb the possibilities for observation of pressor reactions A gradual increase of the stimulus strength to high values was thus seen to cause blood pressure rises and an approximate strength duration curve for the responsible group of afferents could be obtained (curve C in Fig 3) As judged from the experiment illustrated in Fig 3 the depressor responses appear when the hamstring nerves are stimulated in the afferent direction with intensities of about 7 times the threshold for the alpha efferents at pulse durations of 0 1—0 5 msec In the different experiments where such determinations have been performed this value varied between 7 and 13 times the threshold for the efferent fibres Stimulus strengths greater than 50 times this threshold were required to produced pressor effects

In one control experiment attempts were made to determine more accurately the nature of the muscle afferents responsible for the depressor effects Arterial blood pressure and action potentials from dorsal roots were simultaneously recorded in this experiment The threshold intensity for activation of the group I fibres of the hamstring nerve was first determined by means of electrophysiological triphasic recording from the intact ipsilateral posterior root S 1 Fig 4 demonstrates the effects on the arterial blood pressure which were then evoked by an impulse frequency of 9/sec and stimulus intensities corresponding to 1 (A) 2 5 (B) 5 (C) 7 5 (D) 10 (E and F) and 20 (G and H) times the threshold for the group I afferents As judged from the recording of the action potential the group I fibres were maximally activated at 2 5 times threshold Fig 4 shows that the lower intensities do not influence the arterial blood pressure significantly (A—D) but at 10 times threshold a distinct depressor response is discernible (E—F) and at 20 times threshold it amounts to 40 mm Hg (G—H) Repetitive stimulation with trains of 4 impulses at a frequency of 250/sec given 10 times/sec sometimes produced small depressor effects (5—10 mm Hg) at lower stimulus strengths (5—7 5 times threshold for the group I)

The results of the experiments presented above have thus shown that afferent stimulation of muscle nerves with low impulse frequencies induce depressor responses when the strength of the stimuli surpasses 7—13 times threshold for the alpha efferents or about 10 times threshold for the group I afferents These threshold values obtained for the depressor effects as related to the two types of reference fibres can be said to reflect the limit intensities required to induce merely discernible blood pressure responses (5—10 mm Hg) The threshold values for the most excitable units of the afferent fibre population

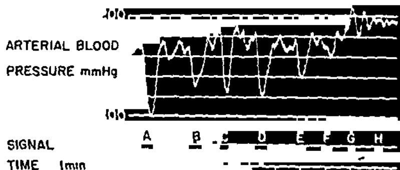


Fig 6 Cat 2 7 kg Chloralose Effects on the arterial blood pressure of various adequate stimuli

A C and D Pinching of the skinned calf muscles on the left side
B Strong passive stretch of the left triceps surae
E Pinching of the right calf muscles not skinned.
F Pinching of a pad on the right hind paw
G Scorching of the skin
H Scorching of a skinned muscle surface

Note that marked blood pressure falls are obtained by mechanical stimulation of muscles especially by squeezing the muscle bellies.
The animal was atropinized curarized and kept under artificial respiration The vagi were cut in the neck and the common carotid arteries occluded

c *Adequate stimulation'*

In 5 experiments attempts were made to investigate the *effects on the* [illegible] [illegible] curarized cat after section of *the* vagal nerves and bilateral occlusion of the common carotid arteries The left hind leg had been skinned between the knee and the *ankle* and the proximal end of the tibia was fixed to the operating table by means of a screw clamp The Achilles tendon was tied and cut distally and a strong passive stretch of the muscle, brought about by pulling the tendon caused a depressor response of 30 mm Hg (B) This effect may be *due to* activation of specific stretch receptors but certainly not of those connected to the group I afferents Sensory stimuli produced by the tendon ligature or the tibial clamp might have contributed more or less to the circulatory response in B of Fig 6 In A, C and D the muscle belly of the left triceps surae was pinched and the arterial blood pressure fell abruptly by 50–60 mm Hg *Pinching* of the *right* calf muscles which were not skinned was also seen to produce a fall in blood pressure (E) but this

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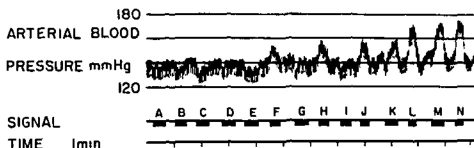


Fig 5 Cat 3 0 kg Chloralose Effects on the arterial blood pressure of afferent stimulation of a cutaneous nerve (left saphenous nerve) at a 'low' and 'high' impulse frequency, a constant pulse duration of 0 2 msec and increasing stimulus strengths

	6 impulses/sec		60 impulses/sec
A	0 5 V	B	0 5 V
C	1 0 V	D	1 0 V
F	2 5 V	F	2 5 V
G	6 V	H	6 V
I	12 V	J	12 V
K	25 V	L	25 V
M	40 V	N	40 V

Slight blood pressure falls (10 mm Hg) are induced at the low frequency and the lower voltages With the high intensities pressor effects are obtained irrespective of the impulse rate used

The animal was atropinized, curarized and kept under artificial respiration The vagi were cut in the neck and the common carotid arteries occluded

A stimulation of 1 0 V and 6 impulses/sec (C) produced a fall in blood pressure of about 10 mm Hg, while the high impulse rate (D) had practically no effect on the blood pressure At 2 5—12 V the low frequency stimulations (E, G, I) were accompanied by minute depressor effects (5—10 mm Hg) while these stimulus strengths produced pressor responses (10—15 mm Hg) at the higher frequency (F, H, J) With 25 and 40 V blood pressure rises were obtained both at low and high impulse rates (K—N) In this particular experiment afferent stimulation of the hamstring nerve produced depressor responses of 40 mm Hg (not shown in the figure)

The results presented above illustrate the difference between the extent of the blood pressure effects obtained in the present study by stimulation of cutaneous and muscle nerves respectively The depressor reactions being the most predominant effect of afferent muscle nerve stimulation were slight or even insignificant when cutaneous nerves were stimulated

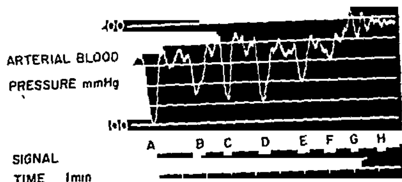


Fig 6 Cat 2 2 kg Chloralose Effects on the arterial blood pressure of various adequate stimuli

A C and D Pinching of the skinned calf muscles on the left side
B Strong passive stretch of the left triceps surae
E Pinching of the right calf muscles, not skinned
F Pinching of a pad on the right hind paw
G Scorching of the skin
H Scorching of a skinned muscle surface

Note that marked blood pressure falls are obtained by mechanical stimulation of muscles especially by squeezing the muscle bellies
The animal was atropinized curarized and kept under artificial respiration The vagi were cut in the neck and the common carotid arteries occluded.

c Adequate stimulation'

In 5 experiments attempts were made to investigate the effects on the arterial blood pressure of different types of 'adequate stimuli' applied to the skin and deeper tissues of the hind limbs Fig 6 shows the results obtained in a chloralose anesthetized curarized cat after section of the vagal nerves and bilateral occlusion of the common carotid arteries The left hind leg had been skinned between the knee and the ankle and the proximal end of the tibia was fixed to the operating table by means of a screw clamp The Achilles tendon was tied and cut distally and a strong passive stretch of the muscle, brought about by pulling the tendon, caused a depressor response of 30 mm Hg (B) This effect may be due to activation of specific stretch receptors but certainly not of those connected to the group I afferents Sensory stimuli produced by the tendon ligature or the tibial clamp might have contributed more or less to the circulatory response in B of Fig 6 In A, C and D the muscle belly of the left triceps surae was pinched and the arterial blood pressure fell abruptly by 50–60 mm Hg Pinching of the *right* calf muscles which were not skinned was also seen to produce a fall in blood pressure (E) but this

was less pronounced than those obtained from the contralateral side In F a slight depressor effect was obtained by pinching one of the pads on the right side while burning the skin of the paw with a hot cautery knife elicited a *pressor response* (G) The blood pressure did not change significantly when the skinned muscles were burnt in the same way (H)

The procedures used for *adequate stimulation* were certainly not very specific but it may be said that pressure or pinching of the muscles have been found to be the most effective methods for *inducing the depressor response* Pressor effects were mainly obtained by nociceptive cutaneous stimuli

d Effects of variations in the general experimental conditions

In the present study 5 experiments were performed in unanesthetized animals decerebrated either by supracollicular transection of the brain stem (2 cats) or by the ischemic method (3 cats) The main interest was focused upon the circulatory effects of afferent muscle nerve stimulation which had been found to produce such marked depressor responses in the chloralose anesthetized cat The two animals with supracollicular decerebration showed *typical blood pressure falls when the central end of the hamstring nerve* was stimulated The maximal response amounted to 60 and 50 mm Hg respectively Fig 7 demonstrates the results obtained in one of these experiments 40—50 min after decerebration and discontinuance of the ether The depressor response is seen to occur at moderate stimulus strengths (0 8—2 V) (A—E in the figure) and it is most pronounced at low impulse frequencies (F —H) At 50 impulses/sec (H) there is even a tendency to a reversal of the response In two of the cats subjected to ischemic decerebration maximal depressor effects of 40 and 70 mm Hg respectively were induced from the hamstring nerves The third animal did not show any *fall in blood pressure* at afferent muscle nerve stimulation This is probably explained by the fact *that the basilar artery in that animal* was ligated at a relatively low level i e within the upper third of the medulla This cat developed in connection with the ligation a marked hyperventilation which might have been due to medullary asphyxia and damage to the bulbar reflex centres mediating the depressor responses (see Chapter V) is likely to have occurred in this experiment

The difference between muscle and skin nerves with regard to their reflex influences on the circulation was as obvious in the *decerebrated animals* as in the anesthetized ones The blood pressure effects observed in animals anesthetized *with Dial* or chloralose urethane were principally of the same character as those obtained in the main chloralose anesthetized group



Fig 7 Cat 3 0 kg Supracollicular decerebration Effects on the arterial blood pressure of afferent stimulation of the hamstring nerve with

A 10 impulses/sec, 0 5 msec and 0 8 V
B 10 impulses/sec, 0 5 msec and 1 2 V
C 10 impulses/sec, 0 5 msec and 1 5 V
D 10 impulses sec, 0 5 msec and 2 0 V
E 10 impulses/sec, 0 5 msec and 2 5 V
F 6 impulses/sec, 0 5 msec and 2.5 V
G 20 impulses sec, 0 5 msec and 2 5 V
H 50 impulses sec, 0 5 msec and 2.5 V

Stimulations of muscle afferents produce typical depressor responses also in the decerebrate preparation

The animal was atropinized, curarized and kept under artificial respiration The vagi were cut in the neck and the common carotid arteries occluded

The depressor responses to afferent somatic nerve stimulation were found to be quantitatively more extensive after experimental procedures (for example, cutting of the baroreceptor fibres) which tended to increase the 'basal' vasoconstrictor fibre activity and to reduce the influence of cardiovascular buffer mechanisms The importance of such factors for the general and regional circulatory adjustments during afferent somatic nerve stimulation will be illustrated and more fully discussed in Chapter IV

Comments

According to LAPORTE, BESSOU and BOUISSET (1960) and SKOGLUND (1960) the depressor effects induced from muscle nerves are due to activation of the group III afferents The blood pressure falls described by these authors were rather small (10—20 mm Hg), and it was therefore considered necessary to ascertain whether possibly other types of afferents contributed to the pronounced depressor responses (40—100 mm Hg) often observed in the present study The results obtained in the series of experiments which was

devoted to this question seem however to be in agreement with those of the above mentioned investigations The depressor effects were thus seen to arise *when the stimulus strength exceeded 10 times threshold for the group I* afferents or 7—13 times threshold for the alpha efferents which corresponds very well with what is known about the characteristics of group III fibres (cf ECCLES and LUNDBERG 1959 PAINTAL 1960)

In the experiment with simultaneous recordings of arterial blood pressure and action potentials from dorsal roots minor depressor reactions (5—10 mm Hg) were occasionally observed at intensities as low as 5 times threshold for the group I fibres especially when repetitive short trains of high frequency stimuli were used This latter finding suggests that fibres within the group II which have been shown to originate mainly in the flower spray endings of the muscle spindles (HUNT 1954 COOPER 1959) might possibly contribute to the depressor influence On the other hand a fraction of the group II fibres seems to be functionally more related to the thinner group III fibres (PAINTAL 1960) and it is therefore possible that the small depressor responses sometimes observed at relatively low stimulus intensities are simply to be explained by this overlap between the two groups Activation of the group II fibres at high frequencies was found by LAPORTE BESSOU and BOUISSET (1960) to induce pressor responses in the decerebrate preparation In the present study with a limited series of decerebrate animals blood pressure *rises were not observed when muscle nerves were stimulated with intensities* below those which induced the typical depressor effects but fairly low impulse frequencies were mostly used The depressor responses to passive stretch of skeletal muscles described by SKOGLUND (1960) may of course be related to an activation of spindle afferents although the tendon loads required to produce the circulatory effects were relatively large (50—300 g) in his experiments

The more remarkable blood pressure falls obtained in the present study by afferent stimulation of muscle nerves must obviously in any case be attributed to activation of fibres within group III The physiological significance of these findings is intimately connected with the functional role and the natural mode of activation of the group III fibres These matters will be discussed in Chapter VI where the experiments with adequate stimulation will also be briefly commented upon

Afferent stimulation of muscle nerves with very high intensities and high impulse frequencies produced pressor effects in these experiments This indicates that a high threshold group of muscle afferents exerts an excitatory action on central cardiovascular neurone pools As judged from the threshold values for the pressor responses (Fig 3 above) and from previous investigations

in this field (von EULER 1947 LAPORTE BESSOU and BOUISSET 1960) these can reasonably be ascribed to an activation of the non medullated C-fibres If precautions were taken to avoid an undue spread of current to adjacent nerves stimulations of muscle nerves with low impulse frequencies (5—15 sec) at these high intensities (10—30 V) were mostly seen to produce blood pressure falls Evidently the depressor action of the thin medullated fibres predominated under these conditions No attempts have been made to study separately the circulatory effects of selective C fibre activation in muscle nerves after previous blockade of the thicker fibres

Concerning the relationship between the blood pressure response and the impulse frequency of the stimulation it was first demonstrated by GRUBER in 1917 and has later been confirmed by other investigators (e g ASHKENAZ 1939 GORDON 1943 LAPORTE BESSOU and BOUISSET 1960) that depressor effects are most likely to occur at low impulse rates The most pronounced blood pressure falls were observed also in the present study when the group III fibres were activated at low frequencies maximal effects being obtained with 5—20 impulses/sec The present results do not seem to warrant the conclusion that a high impul e rate in these fibres should cause a reversal of the blood pressure response since slight depressor effects (10—20 mm Hg) were observed even in the range of 100—400 impulses sec It is evident however that the depressor responses diminish and become less sustained at impulse frequencies above 30 sec even if the stimulus strength is kept so low that an activation of C fibres should not occur

The mechanism behind the inability of the higher frequencies to produce more pronounced depressor reactions is little understood The phenomenon is obviously not due to a transmission failure in the peripheral nerve fibres since these are able to convey impulses at very high frequencies It seems most reasonable to assume that a central nervous mechanism is responsible for the decay of the depressor effects at high frequencies For example a transmission failure may occur in the central synapses at high impulse rates or two different reflex pathways with opposite actions on the cardiovascular centres and with different frequency optima may be available for the afferent impulses It is interesting to note that marked blood pressure falls were induced from the sympatho inhibitory area of the cingulate gyrus at about the same low range of stimulation frequencies (LOFVING 1961 a)

At afferent stimulation of cutaneous nerves pressor responses were the most predominant effects observed and blood pressure falls of 10—20 mm Hg were only occasionally obtained with low impulse frequencies (below 30 sec) and intensities in the range of 0 5—3 V (pulse duration 0 1—0 5 msec) Exact threshold determinations have not been made for skin afferents in

devoted to this question seem however to be in agreement with those of the above mentioned investigations The depressor effects were thus seen to arise when the stimulus strength exceeded 10 times threshold for the group I afferents or 7—13 times threshold for the alpha efferents which corresponds very well with what is known about the characteristics of group III fibres (cf ECCLES and LUNDBERG 1959 PAINTAL 1960)

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LAPORTE, BESSOU and BOUISSET (1960) and by SKOGLUND (1960) seems to be that the tonic sympathetic activity and the cardiovascular reactivity have generally been higher in this series of animals. Thus, in many of the present experiments a definitely increased sensitivity to the sympatho-inhibitory action of the muscle afferents was accomplished by elimination of the influence of one or more of the carotid and aortic baroreceptor areas. In the paper by LAPORTE, BESSOU and BOUISSET the functional importance of the baroreceptors and their possible effects on the circulatory responses studied were not discussed. SKOGLUND stated briefly that elimination of circulatory reflexes from sinus caroticus did not alter the general picture of pressure changes. However, procedures which interfere with normal baroreceptor function can be expected to facilitate the elucidation of the general and regional circulatory effects evoked by somatic afferents. This analytical approach was found especially valuable in the present investigation when the reflex responses of individual vascular beds were studied (Chapter IV). The results reported in this chapter may give an idea about the extent of the sympatho-inhibitory influence that somatic afferents can exert under optimal conditions.

the present study but a comparison of the stimulus characteristics with those used in other investigations on cutaneous nerves (*e g* EVANS 1961) suggests that the small depressor effects are related to thin medullated fibres (gamma delta group) Stimulation with these intensities at higher impulse rates were often seen to produce pressor effects Very strong stimuli which reasonably must have engaged the unmyelinated afferents caused marked blood pressure rises also in the low frequency range These findings are evidently in agreement with the results obtained by LAPORTE and MONTASTRUC (1957)

That depressor responses are more easily obtained from muscle nerves than from cutaneous nerves has been suggested by previous authors (HUNT 1895 TENGWALL 1895) but this view has not been accepted by others (MARTIN and LACEY 1914 RANSON 1921 p 492) As judged from the present results there is such a difference when the effects of cutaneous and muscle nerves of about the same size are compared It may be at least partly explained on the basis of the different composition of cutaneous and muscle nerves with regard to their afferent fibres There is thus a ratio of non medullated to medullated afferents of 3 or 4 to 1 in cutaneous nerves while the corresponding proportion for muscle afferents is about 1 to 1 (RANSON and DAVENPORT 1931 O LEARY HEINBECKER and BISHOP 1935)

According to LAPORTE BESSOU and BOUISSET (1960) the administration of chloralose facilitates the appearance of depressor responses to afferent muscle nerve stimulation Even if the corresponding effects obtained in the present study might sometimes have been enhanced by the action of the anesthetic the experimental results in the five decerebrate cats showed that blood pressure falls of about the same magnitude could be obtained also in this type of preparation The facilitating influence of chloralose described by the French authors might possibly be due to the raised level of sympathetic tonic activity that is generally produced by this agent The influence of anesthesia upon the blood pressure responses to afferent somatic nerve stimulation was examined by MCLENNAN (1961) He found that electrical stimulation of cutaneous nerves in intact unanesthetized curarized (Flaxedil) rabbits always produced a blood pressure rise but this could be converted into a depressor response by the administration of pentobarbitone The significance of this finding seems difficult to evaluate It is possible for instance that massive artificial activation of afferent fibres which may have no primary connections with the vasomotor centres might so affect the emotional balance of a conscious animal that a non specific defence alarm reaction including its cardiovascular components is elicited

The most reasonable explanation for the quantitative differences in the depressor effects of the present study as compared with those described by

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CHAPTER IV

Regional vascular and cardiac adjustments in response to afferent stimulation of somatic nerves

The blood pressure responses evoked by stimulation of somatic afferents must be attributed to changes in cardiac output and/or peripheral vascular resistance brought about by reflex adjustments of the impulse discharge in cardioregulatory and vasomotor nerve fibres To understand the functional significance of these reflexes it is necessary to analyse their cardiovascular response patterns by studying the changes which they induce in the performance of the heart and in the blood supply of different tissues As mentioned in Chapter I the presently available information concerning these problems is rather incomplete

In the experiments presented below heart rate and blood flow in functionally different vascular beds have been recorded in order to elucidate some characteristics of the circulatory responses to afferent somatic nerve stimulation Most of the attention has been directed to the cardiovascular adjustments associated with the *depressor* effects obtained by stimulation of muscle afferents Experimental results with bearing on this problem will be reported in the first section (A) of this chapter Some corresponding observations have been made on the response pattern of the somatic *pressor* reflex and these will be presented under (B)

A Changes of regional blood flows, regional blood volume, and heart rate in depressor responses to afferent muscle nerve stimulation

Results

a Muscle blood flow

Skeletal muscle blood flow was recorded in 37 experiments mostly in combination with venous outflow from one or two other vascular regions Fig 8 illustrates an experiment in which arterial blood pressure and venous outflow from a skeletal muscle region of the left hind limb were recorded The animal was curarized with Flaxedil and an adequate artificial respiration was given Reflex changes in the adrenomedullary secretion were prevented

as previously described (p 19) and atropine had been administered intravenously in a dose of 0 5 mg kg body weight

Stimulation of the central end of the right hamstring nerve with 10 impulses sec 0 5 msec and 2 V was first performed while the vagal nerves were intact and the common carotid arteries patent There was then a reflex fall in blood pressure of about 30 mm Hg (A) The muscle blood flow increased transiently but was soon reduced to less than its original value In order to find out whether this latter reduction was merely a passive phenomenon caused by the reduced perfusion pressure the stimulation of the hamstring nerve was repeated while the arterial inflow pressure was kept constant by adjusting a clamp around the abdominal aorta (B) The stimulation was then seen to produce a more clear cut increase in muscle blood flow although the effect was not sustained throughout the period of stimulation On the basis of the values for blood pressure blood flow and tissue weight given in the figure the reflex changes in the vascular resistance of the muscle region can be approximately estimated in terms of peripheral resistance units (*PRU*) expressed here as mm Hg/ml/min/100 g of tissue (p 19) The increase in muscle blood flow seen in B of Fig 8 can thus be found to correspond to a decrease in the regional vascular resistance from about 40 to about 30 PRU Since the animal was curarized and atropinized and reflex release of catechol amines from the suprarenal glands was prevented the vasodilatation must be ascribed to an inhibition of the tonic activity in the vasoconstrictor fibres to the muscle area studied Actually the reflex dilatations of the muscle vessels induced by stimulation of group III afferents were never seen to be significantly changed by the administration of atropine and there is consequently no reason to assume that the cholinergic vasodilator fibre system is activated in this circulatory response

The fall in blood pressure produced by the hamstring nerve stimulation can be expected to cause a reduced impulse activity in the aortic and carotid baroreceptor afferents The bulbar vasomotor centre would then be partially released from the tonic inhibitory influence of these fibres and the circulatory effects of the nerve stimulation would be counteracted to some extent The regional vasoconstrictor fibre activity as reflected in the blood flow resistance will thus be determined by the balance between the sympatho inhibitory action of the muscle afferents and the tendency to increased spontaneous activity within the vasomotor centre when this is released from the baroreceptor inhibition This may be the explanation for the fact that the regional vascular responses to afferent muscle nerve stimulation have been quantitatively rather variable and sometimes not so easily revealed as long as the buffer mechanisms have been functionally active in a state of normal cardiovascular equilibrium

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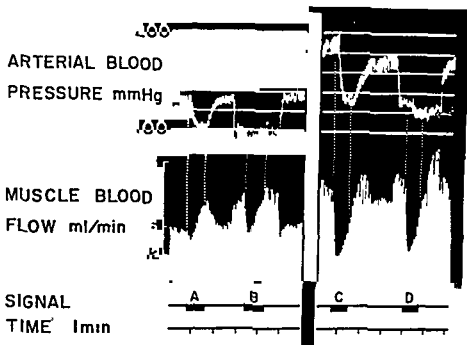


Fig 8 Cat 3 3 kg Chloralose Effects of afferent stimulation of the right hamstring nerve with 10 impulses/sec, 0 5 msec and 2 0 V on arterial blood pressure and muscle blood flow in the left hind limb (approximate tissue weight 170 g)

In A and B the vagal nerves are intact and the carotid arteries patent Bilateral cervical vagotomy and occlusion of the common carotid arteries were done between B and C In B and D the arterial inflow pressure of the muscle region is kept constant before, during and after the nerve stimulation

Note that the general and local sympatho inhibitory responses are enhanced after vagotomy and carotid occlusion, procedures which increase the prestimulatory vascular tone and reduce the buffering capacity of the baroreceptors

The animal was atropinized, curarized and kept under artificial respiration The right suprarenal gland was tied off and the left one denervated

As far as the muscle region is concerned distinct, but in their extent only slight or moderate reflex decreases in the vascular resistance were observed under such circumstances where the buffer mechanisms were intact (B in Fig 8) However, if the buffering capacity of the baroreceptors had been reduced by section of the vagal nerves together with occlusion of the common carotid arteries or section of the sinus nerves stimulation of group III afferents was regularly seen to produce a reflex muscle vasodilatation which was often very pronounced This is shown in the right panel of Fig 8 which illustrates the effects obtained when the central end of the hamstring nerve was stimulated as in A and B but after previous vagotomy and clamping of the carotid arteries

The reflex blood pressure fall was now about 50 mm Hg (C) and the vascular resistance of the muscle region was reduced from about 60 to about 20 PRU as calculated from the record in D where the arterial inflow pressure was kept constant The fact that the reflex vasodilatation was so markedly enhanced in C and D as compared with A and B is due not only to the elimination of the counteracting buffer mechanisms but also to the increased prestimulatory neurogenic tone of the muscle vessels produced by the vagotomy and the carotid occlusion It may be pointed out in this connection that the absolute magnitude of the reflex muscle vasodilatation induced by activation of the group III afferents varied not only in different experiments but also in different phases of the same experiment This could be ascribed mainly to variations in the prestimulatory neurogenic tone of the vessels and the reflex decreases of muscle blood flow resistance obtained seemed thus to be quantitatively roughly proportional to the actual level of this tone Smaller dilator responses in spite of a high tonic vasoconstrictor fibre activity, were seen only under exceptional circumstances as in severe asphyxia The sympatho inhibitory action of the muscle afferents was then obviously unable to compete with the intense sympatho excitatory drive of the asphyxia

b Renal blood flow

Renal and skeletal muscle blood flows were simultaneously recorded in 17 cats and the effects of afferent somatic nerve stimulation on the vascular resistances of the two regions were studied

As judged from the effect of denervation on the venous outflow from the kidney the vessels of this region do not as a rule exhibit any basal neurogenic tone under the present experimental conditions Furthermore even a release of the vasomotor centre from the inhibitory action of the baroreceptors is generally unable to produce any appreciable vasoconstriction in this vascular bed (FOLKOW JOHANSSON and LOFVING 1961 LOFVING 1961 a b) A sympatho [illegible]

[illegible] consequently not be demonstrated in the blood flow recording However if different sympatho excitatory influences are allowed to co operate the renal vasoconstrictor fibres can be more significantly activated and the vessels of the kidney will constrict considerably (FOLKOW, JOHANSSON and LOFVING 1961) Various inhibitory influences of reflex or central nervous origin can then be revealed by the renal blood flow recording

What has been said above is illustrated in Fig 9 which is taken from an experiment where the effects of afferent muscle nerve stimulation on renal and skeletal muscle blood flow were studied Stimulation of the left hamstring

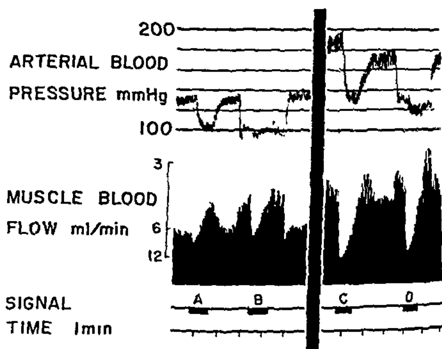


Fig 8 Cat 3 4 kg Chloralose Effects of afferent stimulation of the right hamstring nerve with 10 impulses/sec 0 5 msec and 2 0 V on arterial blood pressure and muscle blood flow in the left hind limb (approximate tissue weight 170 g)

In A and B the vagal nerves are intact and the carotid arteries patent Bilateral cervical vagotomy and occlusion of the common carotid arteries were done between B and C In B and D the arterial inflow pressure of the muscle region is kept constant before during and after the nerve stimulation

Note that the general and local sympatho inhibitory responses are enhanced after vagotomy and carotid occlusion procedures which increase the prestimulatory vascular tone and reduce the buffering capacity of the baroreceptors

The animal was atropinized curarized and kept under artificial respiration The right suprarenal gland was tied off and the left one denervated

As far as the muscle region is concerned distinct but in their extent only slight or moderate, reflex decreases in the vascular resistance were observed under such circumstances where the buffer mechanisms were intact (B in Fig 8) However if the buffering capacity of the baroreceptors had been reduced by section of the vagal nerves together with occlusion of the common carotid arteries or section of the sinus nerves stimulation of group III afferents was regularly seen to produce a reflex muscle vasodilatation which was often very pronounced This is shown in the right panel of Fig 8 which illustrates the effects obtained when the central end of the hamstring nerve was stimulated as in A and B but after previous vagotomy and clamping of the carotid arteries

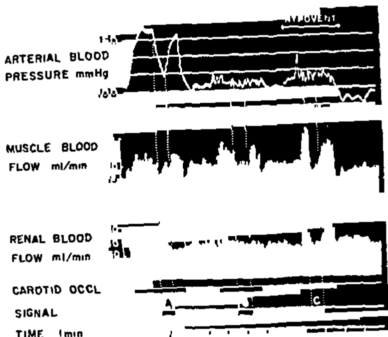


Fig 9 Cat 3 2 kg Chloralose Effects of afferent stimulation of the left *hamstring nerve* (10 impulses sec, 1 msec and 3 0 V) on blood pressure and blood flow in the skeletal muscles of the right hind limb and in the left kidney Stimulations performed during three different periods *of bilateral carotid* occlusion In the last two thirds of the recording the arterial inflow pressure of the regions studied is kept constant during the carotid occlusions and nerve stimulations

Note that the muscle vessels constrict when the carotid arteries are occluded and dilate in connection *with the depressor* responses to hamstring nerve stimulation (A, B, C) Carotid occlusion produces a significant vasoconstriction in the kidney only when the animal *is subjected also* to a moderate hypoventilation When a renal neurogenic vascular tone has thus been established the muscle nerve stimulation produces a reflex vasodilatation in this region also (C)

The animal was atropinized, curarized and kept under artificial respiration and the vagal nerves were cut in the neck *The right adrenal gland* was tied off and the left one denervated Approximate weight of the muscle region 160 g kidney weight 10 g

impulse discharge in the vasoconstrictor fibres to the muscles by at least 1–2 impulses sec If a corresponding activation of the sympathetic fibres to the kidney had taken place at the same time, this should have manifested itself as a definite increase *of the renal* vascular resistance The renal vaso constriction observed in Fig 9 when the carotid arteries were occluded during

nerve during periods of carotid artery occlusion (A and B) produced a fall in blood pressure of 50 mm Hg accompanied by a decrease in the vascular resistance of the skeletal muscles In the first section of Fig 9 the blood flow of the kidney seemed to change passively with the shifts in blood pressure produced by the carotid occlusion and the hamstring nerve stimulation In the second period of carotid occlusion and in the stimulation B where the arterial inflow pressure was kept constant there were no definite changes in the renal blood flow The flow resistance of the muscle vessels increased from about 17 to 27 PRU when the carotid arteries were occluded and it returned again to its original value during the hamstring nerve stimulation (B) The response pattern is very different in the third section of the record in Fig 9 where the carotid arteries were clamped and the hamstring nerve stimulated during a period of moderate hypoventilation The arterial inflow pressure of the regions studied was kept constant by partial compression of the proximal abdominal aorta Under these circumstances occlusion of the common carotid arteries produced a reflex constriction of the renal vessels also corresponding to an increase of some 30 per cent in vascular resistance (from 0 24 to 0 32 PRU) The renal vasoconstrictor fibre activity which had thus been built up is seen to be reflexly inhibited by afferent impulses in the hamstring nerve (C) The reflex vascular reactions of the muscle region in C have the same major characteristics as in B even if they are quantitatively more pronounced

As far as the reflex vascular responses to the occlusions of the carotid arteries are concerned the results illustrated in Fig 9 are in agreement with those reported by FOLKOW JOHANSSON and LOFVING (1961) It was suggested by these authors that the central neurone pools controlling the impulse discharge in the vasoconstrictor neurones of functionally different tissues were characterized by differences in excitability or activation threshold Such a difference between the central structures regulating the activity of the vasoconstrictor fibres to muscle and kidney respectively may explain the change of the response observed in Fig 9 when the last two periods of carotid artery occlusion are compared The vasoconstrictor fibres to the skeletal muscles were thus activated to a marked extent when the vasomotor centre was merely influenced by the change in baro and chemoreceptor activity produced by the carotid occlusion An additional excitatory influence here represented by a moderate asphyxia was evidently required to engage the renal vasoconstrictor fibres

On the basis of the frequency response curves for the muscle and renal vessels obtained by CELANDER (1954) it can be assumed that the carotid artery occlusion preceeding B in Fig 9 implied an average increase of the

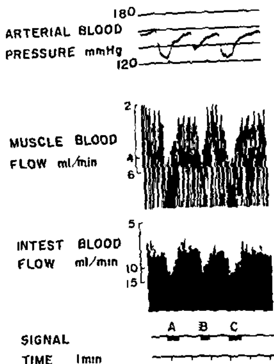


Fig 10 Cat 2 5 kg Chloralose Effects of afferent stimulation (10 impulses/sec, 0 5 msec and 1 0 V) of the right hamstring nerve (A) the right quadriceps nerve (B) and the right brachial plexus (C) on blood pressure and blood flow in muscle (left hind limb) and intestine

Note that a reflex vasodilatation is obtained in both regions and that the response pattern induced by the three different nerves is practically the same

The animal was atropinized curarized and kept under artificial respiration The vagal nerves were cut in the neck and the common carotid arteries occluded Approximate weight of the muscle region 120 g and of the intestinal section 20 g

of the afferent fibres This has been a constant finding in these experiments, and there have been no observations to indicate a difference in the sympatho inhibitory response pattern produced by flexor or extensor afferents or by afferents at different segmental levels

As previously discussed for renal and muscle vessels the reflex vasodilatation in the intestine can be attributed to an inhibition of tonic vasoconstrictor fibre activity Reflex changes in intestinal motility or secretion could,

a period of hypoventilation would — again with reference to CELANDER's study — correspond to a mean vasoconstrictor fibre discharge of approximately 1—2 impulses/sec

The reflex vascular reactions produced by the hamstring nerve stimulation in B of Fig 9 would easily lead to the erroneous conclusion that the activity of the vasoconstrictor neurones to the muscle vessels is *selectively* inhibited by afferent impulses in the group III fibres from muscle However, it is evident from the vascular effects observed in C, that inhibitory reflex connections between the group III afferents and the renal vasoconstrictor neurones do exist and exert their vascular effects whenever a tonic constrictor fibre activity is present

c Intestinal blood flow

The effects of afferent muscle nerve stimulation upon intestinal and muscle blood flow were studied in 8 experiments The results obtained in one of them, representative for the group, are demonstrated in Fig 10 which also illustrates the depressor response pattern evoked by stimulation of group III fibres in *different* somatic nerves The neurogenic tone of the two vascular beds had been increased by vagotomy and bilateral occlusion of the common carotid arteries, and their vascular resistance was consequently rather high (muscle about 75 PRU, intestine about 4 3 PRU) The right hamstring nerve (A), the right quadriceps nerve (B) and the right brachial plexus (C) were stimulated in the afferent direction with 10 impulses/sec, 0 3 msec and 1 0 V On the basis of the results presented in the previous chapter, it is reasonable to assume that the circulatory effects produced by stimulation of the brachial plexus with these stimulus characteristics were mainly due to activation of thin medullated muscle afferents within this mixed nerve bundle

Blood pressure falls of 20—30 mm Hg were obtained in the three different stimulation periods of Fig 10 More pronounced effects could be elicited by increasing the voltage, but submaximal stimuli were assumed to be more suitable for the demonstration of possible differences in the reflex patterns produced by the three different nerves which represent afferents from flexor and extensor muscles and from different segmental levels The depressor responses in A B and C were however all accompanied by a decrease in vascular resistance in booth muscle and intestine which was quantitatively correlated with the fall in blood pressure As calculated from A in the figure, the blood flow resistance in the muscle region decreased from 75 to 40 and in the intestinal region from 4 3 to 2 1 PRU approximately

The regional vascular responses were not influenced by the site of origin

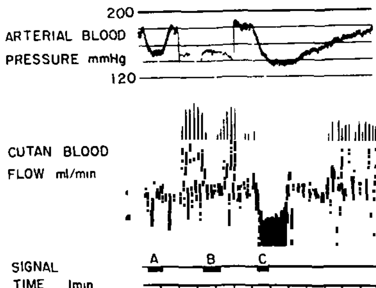


Fig 11 Cat 2 9 kg Chloralose urethane Effects on blood pressure and cutaneous blood flow of afferent stimulation of the hamstring nerve with 6 impulses/sec, 0 5 msec and 2 5 V (A and B) and of the hypothalamic 'heat loss area' with 60 impulses/sec, 1 5 msec and 4 V (C) In B the arterial inflow pressure is kept constant before, during and after the stimulation

The cutaneous vasodilatation accompanying the 'somatic depressor response' is slight as compared with th ...

gland was tied off and the left one denervated

anterior commisure and the optic chiasma (MAGOUN *et al* 1938, FOLKOW, STROM and UVNAS 1949, STROM 1950, ANDERSSON, GRANT and LARSSON 1956 etc) The investigations by FOLKOW, STRÖM and UVNÄS and by STRÖM showed that a pronounced vasodilatation can be produced in the cat's paw by local warming in this area

Fig 11 shows the results obtained in one of the present experiments where cutaneous blood flow was recorded from the left hind paw Afferent stimulation of the right hamstring nerve produced a blood pressure fall of about 30 mm Hg and the cutaneous blood flow resistance decreased to about 80 per cent of its control value (A, B) In C *of Fig* 11 a region of the anterior hypo thalamus just above the optic chiasma was stimulated bilaterally with 60

4

of course be accompanied by secondary shifts in the venous outflow from the jejunal loop but such effects are unlikely to influence significantly the vascular events in the vagotomized and atropinized animal The adrenal glands and their innervation were left intact in most of the experiments with recordings of intestinal blood flow in order to avoid an undue damage of the splanchnic fibres to the gut As illustrated in Fig 10 the vascular reactions had such an immediate onset in both muscle and intestine that they must be neurogenically induced and not primarily related to reflex changes in the adrenomedullary output of catechols Further the same distinct pattern of the intestinal vascular response as that shown in Fig 10 was observed in experiments where the adrenals had been excluded as described on p 19

The results have thus indicated that impulses in the muscle afferents which evoke depressor responses also produce an inhibition of the tonic discharge in the vasoconstrictor fibres to the intestinal vascular bed The effects ob tained were quantitatively enhanced by procedures (*e g* carotid occlusion) which increased the prestimulatory neurogenic tone of the intestinal vessels A moderate but clear cut vasodilatation was however often observed in this area even when muscle afferents were stimulated in animals with intact carotid baroreceptor mechanisms

d Cutaneous blood flow

The effects of afferent somatic nerve stimulation on cutaneous blood flow were studied in 8 experiments with recordings of venous outflow from the large saphenous vein In 4 of these experiments muscle blood flow was simul taneously recorded Depressor responses to activation of muscle afferents were found to be accompanied by a reflex vasodilatation in the skin of the paw indicating an inhibition of the tonic vasoconstrictor fibre activity to this region also However cutaneous vascular resistance was never markedly reduced by the muscle nerve stimulation even if the prevailing neurogenic tone of these vessels was high as judged from the effect of a subsequent de nervation In this respect the skin seemed to differ from the other vascular regions examined in this study To rule out the possibility that environmental or other nonspecific factors were responsible for the inability of the cutaneous vessels to dilate to a greater extent during a somatic depressor response it was considered of interest to compare this inhibitory effect on the cutaneous vasoconstrictor fibre activity with that produced by stimulation of the hypo thalamic heat loss area A variety of reactions like sweating panting cuta neous vasodilatation etc all designed for the dissipation of heat can be evoked by thermal or electrical stimulation of this region situated between the

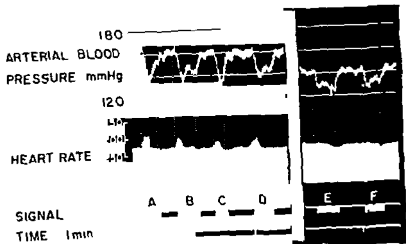


Fig 12 Cat 3 0 kg Chloralose Effects of afferent hamstring nerve stimulation (10 im pulses/sec, 0 5 msec and 1 5 V) on blood pressure and heart rate before (A—D) and after (E—F) atropinization

The initial bradycardia accompanying the reflex blood pressure falls is abolished by the administration of atropine

To investigate the possible reflex responses of the venous vascular section in the circulatory adjustments induced from somatic afferents, the reactions of the resistance and capacitance vessels of the intestine were simultaneously studied in a few experiments using a method described by FOLKOW, LUNDGREN and WALLENTIN (1962) It was found that a blood pressure fall induced by stimulation of 'somatic depressor afferents' was accompanied not only by a decrease of the blood flow resistance in this region but also by a dilatation of the capacitance vessels as revealed by an immediate increase of tissue volume The experiments are too preliminary to permit a definite quantitative evaluation of the regional accumulation of blood during a 'somatic depressor effect' but it seems clear that a relaxation of the capacitance vessels is one component of the reflex response

f Heart rate

In order to find out whether a vagal ... cular response pattern induced by ... heart rate was recorded in 6 exp... hamstring nerve was stimulated before and after atropinization or vagotomy Fig 12 shows the results obtained in one of these animals The heart frequency was registered

impulses/sec 1 msec and 4 V The vessels of the skin were now markedly dilated and their flow resistance was reduced to less than 20 per cent of the prestimulatory value This seemed to correspond to a total inhibition of tonic vasoconstrictor fibre activity to this region since a subsequent extirpation of the left lumbar sympathetic chain caused nearly exactly the same decrease in flow resistance (not shown in the figure) There was also a blood pressure fall in C of Fig 11 which may mean that the cardiovascular buffer mechanisms were so impaired by the vagotomy and the carotid artery occlusion that they were unable to compensate for the cutaneous vasodilatation possibly including a pooling of blood in superficial veins

The experiments with *recordings of venous outflow from the skin* have thus indicated that the tonic activity of the vasoconstrictor neurones to the vessel of this region is also inhibited by activation of muscle afferents This effect seems however to be relatively weak as compared with the inhibitory action exerted by the hypothalamic heat loss area The cutaneous blood vessels did not dilate to any marked extent even when the blood pressure fall obtained by afferent muscle nerve stimulation was greater than that seen in Fig 11 and was accompanied by a considerable increase of muscle blood flow indicating a pronounced inhibition of sympathetic activity It seems reasonable to assume that the vasomotor fibres to the skin at least those to the arteriovenous anastomoses of the pads are preferentially controlled by nervous structures related to the regulation of body temperature and that they are comparatively less engaged in more generalized circulatory adjustments

e Effects on the venous vascular section

The experiments presented above have illustrated the effects of afferent muscle nerve stimulation on the neurogenic tone of the resistance vessels in four different parallel coupled vascular circuits The resistance vessels constitute however only one though admittedly important section of these circuits Each of them contains also other functionally different series coupled vascular sections such as precapillary sphincter vessles exchange vessels capacitance vessels etc (see Folkow 1960 b Mellander 1960) of which the capacitance vessels are of special interest with regard to the nervous control of the circulation Sympathetic vasoconstrictor fibres are distributed to the capacitance section which is localized *mainly on the venous* side of the vascular beds Changes in the impulse activity of these fibres will influence the regional blood content and thus contribute to the mechanisms of pooling and mobilization of *blood* (Folkow 1960 b Mellander 1960)

the existence of a local or segmental organization of the somatic depressor reflex with regard to the vascular effects induced In animals deprived of their cardiovascular buffer mechanisms the reflex vasodilatation obtained in the separate vascular beds was found to be quantitatively dependent upon the prevailing neurogenic tone of the vessels For example the skeletal muscle area which is usually characterized by a high neurogenic vascular tone after elimination of the baroreceptor mechanisms (LÖFVING 1961 a b) was consequently in most cases seen to present an especially pronounced reflex decrease of vascular resistance during a somatic depressor response' There was no evidence for a reflex activation of the sympathetic vasodilator fibres to the muscles since the induced changes of the blood flow in this region were the same before and after administration of atropine

Renal and intestinal vessels were also found to dilate in proportion to their actual degree of constrictor fibre tone while the vessels of the skin including the specialized vasculature of the pads showed only moderate or slight dilatations even if their original tone was high Due to the steepness of the frequency response curve of the cutaneous vascular bed (CELANDER and FOLKOW 1953 CELANDER 1954) clear cut reflex dilatations can be expected to occur in this region even if the tonic activity of the constrictor fibres is only slightly inhibited The vasoconstrictor fibre activity to this tissue appears to be dominated by thermoregulatory nervous mechanisms and is therefore relatively little affected by more generalized vasomotor reflexes including the somatic depressor responses It may be recalled for instance that on an exposure of the organism to an intense heat load the blood pressure and blood volume regulating mechanisms may be unable to produce any significant reflex constriction of the cutaneous vessels even if a circulatory collapse is imminent

The fact that the group III fibres have been shown to exert a generalized inhibitory influence upon the tonic vasoconstrictor fibre activity indicates that this somatic depressor reflex is not *primarily* organized for differentiated vasomotor adjustments i e for the purpose of redistribution of blood from one vascular area to another It is obvious however from some of the experimental results presented above that this basic pattern of generalized vasodilatation may be largely modified by a number of factors The influence of regional differences in the prevailing neurogenic vascular tone and in the characteristics of the frequency response curves of the neuro effectors has already been mentioned It is also obvious that the local temperature and the state of metabolic activity in an organ will affect the possibilities for its vessels to respond to changes in vasoconstrictor fibre discharge Even if the vascular response pattern of a vasomotor reflex is thus primarily determined by the arrangement of the neuronal connections in the central nervous system

by means of a pulse recorder operating an ordinate writer. With the carotid and aortic baroreceptor mechanisms intact, afferent stimulation of the left hamstring nerve produced only moderate depressor responses (20—30 mm Hg). These were accompanied, or rather preceded by a temporary decrease in heart rate from 230 to about 200 beats per min (A—D). This bradycardia had a rapid onset but was usually of a short duration, possibly due to compensatory cardioregulatory reflexes provoked by the blood pressure fall. After the administration of atropine 0.5 mg/kg this initial bradycardia did not appear when the hamstring nerve was stimulated (E—F) and it can therefore be ascribed to a reflex activation of vagal cardio inhibitory nerve fibres. This *initial* vagal bradycardia was a constant observation but was generally of a moderate extent, a reduction of the pulse rate by about 20 % being the most common finding. Marked reflex changes in the heart rate are, however, only exceptionally observed in cats.

In animals where *an intensified sympathetic activity* had been produced by bilateral cervical vagotomy and carotid artery occlusion, the pronounced *depressor responses induced by afferent muscle nerve stimulation were often* found to be accompanied by an immediate but moderate decrease in pulse rate. This phenomenon can evidently be ascribed to a reflex inhibition of the impulse discharge in sympathetic accelerans fibres.

Comments

Regional vascular responses associated with the depressor effects of afferent somatic nerve stimulation were studied with different techniques in some early investigations (see McDowall 1956, pp. 75—89). The experimental results presented by different authors were, however, rather divergent and did not lead to a general concept of the circulatory *response pattern or to a* functional evaluation of the reflex phenomena. As mentioned in Chapter I, little attention has subsequently been paid to the circulatory *effects initiated* from somatic sensory nerves and the response patterns are still incompletely known (Uvnäs 1960 b, p. 1145).

The experiments presented above have shown that the depressor responses to afferent muscle nerve stimulation are largely due to a reduced peripheral vascular resistance brought about by a generalized inhibition of the tonic activity in the sympathetic vasoconstrictor fibres. The cardiovascular effects initiated by stimulation of muscle afferents *are thus closely similar to the* sympatho-inhibitory responses obtained from cortico-hypothalamic structures and from vagal or carotid sinus afferents (Folkow, Johansson and Öberg 1959, Löfving 1961 a). The present results have not provided any evidence for

ing the quantitative engagement of renal and muscle vessels in this somatic pressor reflex as compared with other types of pressor responses

Fig 13 shows a recording of arterial blood pressure and venous outflow from the right kidney The vagal nerves were cut and a reflex release of adrenomedullary hormones was prevented as described on p 19 The animal was curarized with Flaxedil and a sufficient artificial respiration was given The effect of an afferent stimulation of the brachial plexus with 50 impulses sec, 0 5 msec and 20 V is shown in A of Fig 13 The blood pressure increased from about 120 to about 160 mm Hg and the renal blood flow was simultaneously reduced to less than half of its original value although this effect was not sustained throughout the stimulation period A bilateral occlusion of the common carotid arteries (B) produced about the same increment in blood pressure as was obtained in A but little or no change could now be observed in the renal blood flow Repetition of the somatic nerve stimulation during this period of carotid occlusion (C) caused a renal vasoconstriction greatly intensified as compared with A and accompanied by a further rise in blood pressure The carotid clamps were removed at D in the figure The experimental procedures performed in A—D were repeated again in E—H of Fig 13 but now the arterial inflow pressure of the kidney was kept constant by partial compression of the proximal abdominal aorta Stimulation of the brachial plexus (E) again produced a marked increase in renal vascular resistance (from 0 3 to 0 7 PRU) while the carotid occlusion (F) produced only a moderate constriction of the renal vessels (blood flow resistance from 0 3 to 0 4 PRU) Afferent nerve stimulation performed while the carotids were still occluded (G) caused an increase of the vascular resistance of the kidney to a very high value (1 7 PRU)

Fig 13 illustrates the striking difference between the renal vasoconstrictor response induced by afferent somatic nerve stimulation and that produced by occlusion of the common carotid arteries In spite of the fact that these separate procedures caused nearly exactly the same rise in blood pressure (A and B in the figure) the accompanying reflex increase in renal vascular resistance produced by the somatic pressor afferents was about four times greater than that produced by the carotid occlusion An estimation based on CELANDER's (1954) results previously referred to indicates that the former reflex effect corresponds here to an average increase of renal vasoconstrictor fibre discharge by about 3 impulses sec while in the latter response it should be less than 1 impulse sec This quantitative difference which has been observed repeatedly in these experiments indicates per se that the discharge pattern of the vasomotor fibre system may differ considerably in these two pressor reflexes The vasoconstrictor fibres to the kidney were thus markedly engaged

as revealed in analytical investigations it can be largely modified by other nervous influences of central or reflex origin and by peripheral environmental factors

The reduced peripheral resistance is apparently not the only factor responsible for the fall in blood pressure obtained by stimulation of muscle afferents since preliminary experiments in this series have indicated that a pooling of blood may occur as a result of inhibition of the tonic activity in the sympathetic vasoconstrictor fibres to capacitance vessels It was further demonstrated that a vagal bradycardia and an inhibition of the discharge in sympathetic accelerans fibres are components of the response pattern Therefore it is reasonable to assume that the cardiac output may be reduced in a somatic depressor reaction', especially if hydrostatic factors should happen to enhance the pooling effect of the reflex dilatation within the capacitance vascular section, for this could markedly reduce the venous return to the heart

The extent of the depressor responses induced by activation of group III afferents must be intimately dependent on the prevailing equilibrium of nervous cardiovascular control It is apparent from the present results that the depressor effects are greatly enhanced under circumstances where the tonic sympathetic activity is high and, in addition the buffering capacity of the cardiovascular 'proprioceptors' impaired A situation of this kind must in fact occur in the intact organism if the buffer reflexes are engaged in responses directed towards compensation for peripheral pooling of blood or blood loss Then the vasoconstrictor fibre activity would be high and the cardiovascular 'proprioceptors' less capable of counteracting other reflex sympatho inhibitory influences A sudden intense activation of somatic depressor afferents' under such circumstances can be expected to produce really drastic changes in the circulatory homeostasis even to the extent that a circulatory collapse' may ensue

B Observations on changes of regional blood flows in the 'somatic pressor reflex'

Results

The present experiments have shown that an increased vascular resistance can be induced in muscle skin intestine and kidney by stimulating high threshold afferents in cutaneous or mixed somatic nerves The results reported in this section will be mainly confined however, to some observations concern

in the 'somatic pressor reflex They were on the other hand only slightly activated by clamping of the carotid arteries provided no other excitatory influence was affecting the vasomotor centre Since afferent somatic nerve stimulation caused a still more pronounced effect on the renal blood flow if performed during a period of carotid occlusion (E and G in Fig 13) the excitability of the renal vasoconstrictor neurones was obviously increased also by the latter procedure

Fig 14 shows the effects of afferent somatic nerve stimulation and carotid artery occlusion upon the renal and skeletal muscle blood flows when these parameters were simultaneously studied in an atropinized 'adrenalectomized cat The animal was slightly hypoventilated a procedure which had previously been found to increase the reactivity of the renal vasoconstrictor neurones (p 44) A C and E of Fig 14 represent stimulations of the central end of the brachial plexus with 50 impulses/sec 0 5 msec and 20 V, while B and D—F represent periods of carotid artery occlusion The relative degree of renal vasoconstriction shows the same principal pattern of response to the two different experimental procedures as discussed in connection with the previous figure, although here the effect of carotid occlusion is somewhat larger due to the hypoventilation The afferent somatic nerve stimulation produced only a temporary reduction in the *skeletal muscle* blood flow as seen in A and C of Fig 14 Occlusion of the common carotid arteries on the contrary, was accompanied by a more pronounced and in addition sustained constriction of the resistance vessels in this region as shown in B and D—F When the excitatory effect of somatic nerve stimulation was added to that of the carotid sinus reflex (E) no further change in the muscle blood flow was observed The fact that a further decrease in muscle blood flow was not obtained here may be related to the frequency response curve of the neuro effectors since the vascular resistance has already increased close to the extent which is maximally possible for a vasoconstrictor fibre activation to this region Therefore it does not necessarily indicate that an additional increase in the discharge rate of the vasoconstrictor neurones to the skeletal muscles did not occur A comparison of the muscle blood flow responses to stimulation of 'somatic pressor afferents (A C) and to carotid artery occlusion (B D—F) respectively, shows that the vasoconstrictor fibres to this region were activated to a much greater extent by the latter procedure than by the former one

The circulatory response patterns of the two different pressor *reflexes* — *one* induced from cardiovascular proprioceptors the other mainly from somatic C fibres — have thus been found to be quantitatively rather dissimilar as far as the vascular effects in muscle and kidney are concerned It is here of interest to recall that the composition of the adrenal catechol secretion with regard to

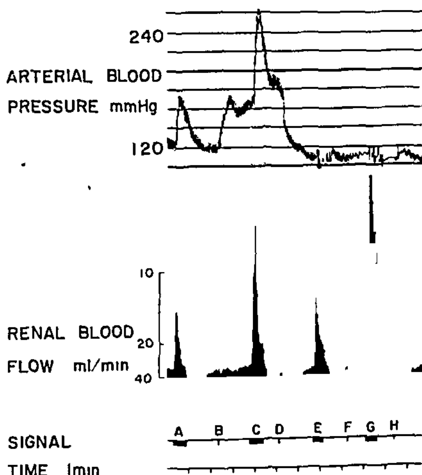


Fig 13 Cat 3 3 kg Chloralose Effects of stimulations of 'somatic pressor afferents' and of carotid artery occlusions on blood pressure and renal blood flow

A, C, E and G Afferent stimulation of the brachial plexus with 50 impulses/sec, 0 5 msec and 20 V

B–D and F–H Bilateral occlusion of the common carotid arteries Arterial inflow pressure is kept constant in E–H

Note that the somatic nerve stimulation causes a blood pressure rise accompanied by a marked renal vasoconstriction (A and E) while the carotid occlusion, which produces about the same rise in blood pressure (B), gives only a slight constriction in the kidney The effect of the 'somatic pressor afferents' is enhanced by the carotid occlusion (C and G)

The animal was curarized and kept under artificial respiration and the vagal nerves were cut in the neck The right adrenal gland was tied off and the left one denervated Kidney weight 12 g

in the somatic pressor reflex They were on the other hand only slightly activated by clamping of the carotid arteries provided no other excitatory influence was affecting the vasomotor centre Since afferent somatic nerve stimulation caused a still more pronounced effect on the renal blood flow if performed during a period of carotid occlusion (E and G in Fig 13) the excitability of the renal vasoconstrictor neurones was obviously increased also by the latter procedure

Fig 14 shows the effects of afferent somatic nerve stimulation and carotid artery occlusion upon the renal and skeletal muscle blood flows when these parameters were simultaneously studied in an atropinized adrenalectomized cat The animal was slightly hypoventilated a procedure which had previously been found to increase the reactivity of the renal vasoconstrictor neurones (p 44) A C and E of Fig 14 represent stimulations of the central end of the brachial plexus with 50 impulses/sec 0 5 msec and 20 V while B and D—F represent periods of carotid artery occlusion The relative degree of renal vasoconstriction shows the same principal pattern of response to the two different experimental procedures as discussed in connection with the previous figure although here the effect of carotid occlusion is somewhat larger due to the hypoventilation The afferent somatic nerve stimulation produced only a temporary reduction in the *skeletal muscle* blood flow as seen in A and C of Fig 14 Occlusion of the common carotid arteries on the contrary was accompanied by a more pronounced and in addition sustained constriction of the resistance vessels in this region as shown in B and D—F When the excitatory effect of somatic nerve stimulation was added to that of the carotid sinus reflex (E) no further change in the muscle blood flow was observed The fact that a further decrease in muscle blood flow was not obtained here may be related to the frequency response curve of the neuro effectors since the vascular resistance has already increased close to the extent which is maximally possible for a vasoconstrictor fibre activation to this region Therefore it does not necessarily indicate that an additional increase in the discharge rate of the vasoconstrictor neurones to the skeletal muscles did not occur A comparison of the muscle blood flow responses to stimulation of somatic pressor afferents (A C) and to carotid artery occlusion (B D—F) respectively shows that the vasoconstrictor fibres to this region were activated to a much greater extent by the latter procedure than by the former one

The circulatory response patterns of the two different pressor reflexes — one induced from cardiovascular proprioceptors the other mainly from somatic C fibres — have thus been found to be quantitatively rather dissimilar as far as the vascular effects in muscle and kidney are concerned It is here of interest to recall that the composition of the adrenal catechol secretion with regard to

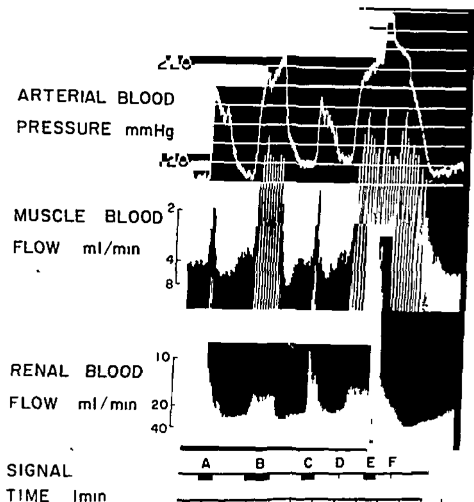


Fig 14 Cat 3 3 kg Chloralose Effects of stimulation of somatic pressor afferents and of carotid artery occlusion on blood pressure, muscle and renal blood flow

A C and F *Afferent stimulation of the brachial plexus with 50 impulses/sec, 0 5 msec* and 20 V

B and D—F Bilateral occlusion of the common carotid arteries

Note that the muscle vessels, which are markedly constricted in connection with the carotid occlusion show only a moderate, transient response in the somatic pressor reflex The renal vessels, on the other hand *are relatively more engaged in the latter reflex* adjustment

The animal was atropinized, curarized and slightly hypoventilated The vagal nerves were cut in the neck The right adrenal gland was tied off and the left one denervated Approximate weight of the muscle region *170 g Kidney weight 12 g*

the relative amounts of adrenaline and noradrenaline, had also been found to be different when the sympathetic fibres to the adrenal medullae were reflexly activated by carotid occlusion and by stimulation of 'somatic pressor afferents', respectively (VON EULER and FOLKOW 1953) The output of adrenaline was proportionally higher in the latter response The hormonal factor was not, however, engaged in the vascular adjustements described above, since the present experiments were performed on 'adrenalectomized' animals but the findings of VON EULER and FOLKOW provide a further illustration of the differences between the two autonomic response patterns

The observation that the muscle vessels were markedly constricted when the carotid arteries were occluded, while the renal blood flow was relatively little affected by this procedure, is quite in accordance with the pattern of circulatory response to baroreceptor inactivation which has previously been described by LOFVING (1961 a, b) As mentioned, the renal vasoconstriction was regularly more pronounced in the pressor response to afferent somatic nerve stimulation, while the skeletal muscle area showed rather variable changes in blood flow A relatively moderate and transient increase in the vascular resistance of the muscle region, as in A and C of Fig 14, was mostly observed in the atropinized animals, but occasionally a more sustained vaso constrictor response was seen Attempts have also been made to find out whether the cholinergic vasodilator fibres to the skeletal muscle are possibly activated in the 'somatic pressor reflex' It was sometimes observed that the blood flow resistance in the muscle region actually decreased in association with the blood pressure rise and this reflex dilatation was then partially blocked by the administration of atropine, but the results have not been quite conclusive in this respect

The reflex changes in regional blood flow, as studied in these experiments, did not vary qualitatively if the pressor response was elicited from contralateral or ipsilateral somatic nerves of different segmental origin When lumbar dorsal roots were stimulated in the afferent direction while venous outflow was simultaneously recorded from one hind limb and from the ipsilateral forelimb there was no evidence of any local or segmental organization of the vascular responses induced by somatic pressor afferents' A circulatory adjustment in conformity with the 'Lovén reflex' has thus not been observed in the present study

In experiments where intestinal or cutaneous blood flow were recorded, it was generally found that a clear-cut vasoconstriction occurred in these vascular beds when somatic pressor afferents' were stimulated

Comments

Stimulation of afferent spinal nerves with high stimulus strength and high impulse frequency can obviously produce a widespread activation of the sympathetic vasoconstrictor fibres but the discharge pattern was found to differ considerably from the pressor reflex adjustments produced by carotid occlusion The fact that the vessels of the skeletal muscles were relatively little engaged in the 'somatic pressor response' — and that there might occasionally even have been an 'active vasodilatation in this region — may be important for the evaluation of the physiological significance of this circulatory reflex It is of interest in this connection that the defence alarm reaction which includes an activation of the cholinergic vasodilator fibre system to the skeletal muscles has been reflexly evoked in high decerebrate cats by various afferent influences among them nociceptive cutaneous stimuli (ABRAHAMS HILTON and ZBROZYNA 1960 a b) Stimulation of the diencephalic vasodilator area' produces besides the active' muscle vasodilatation an intense activation of the vasoconstrictor fibres to the vessels of the skin intestine (LINDGREN 1955) and kidney (FEIGL JOHANSSON and LOFVING 1962 FEIGL and LOFVING 1962) and of the sympathetic fibres to the heart (ROSÉN 1961) The circulatory response pattern induced from this hypothalamic integrative station of the defence alarm reaction presents certain similarities to the circulatory adjustment produced by stimulation of the somatic pressor afferents which are probably nociceptive (see Chapter VI) It seems likely that these afferents evoke their circulatory effects by influencing cardiovascular centres at different levels of the central nervous system A widespread vasoconstrictor response is evidently mediated by reflex connections at the bulbospinal plane and this response pattern may dominate in anesthetized animals since the bulbar cardiovascular centres are relatively more resistant to anesthetic agents than are the higher levels of the central nervous system It is known that a primitive sort of defence behaviour occurs in bulbospinal preparations (KELLER 1932) but it is also clear that such reactions become more complex and purposeful in intact or decorticate animals The cardiovascular response pattern produced by the somatic pressor afferents in the present experiments might represent a kind of lower level framework for the circulatory adjustment associated with the defence behaviour This primitive pattern may normally be overcome by the more differentiated diencephalic pattern which includes an intense activation of the sympathetic vasodilator fibres to the skeletal muscles If such a reflex organization is assumed the above mentioned occasional signs of active' muscle vasodilatation during a somatic pressor response may indicate that the diencephalic reflex centres were sometimes activated by the afferent nerve stimulations in spite of the depressing influence of anesthesia

CHAPTER V

Spinal pathways and bulbar structures engaged in the 'somatic depressor and pressor reflexes'

The experiments presented in this chapter will mainly be concerned with the central nervous arrangement of the depressor reflex induced from muscle afferents although some observations on the spinal pathways related to the somatic pressor reflex will also be included

As mentioned in Chapter I the relative importance of propriospinal and bulbar reflex connections for the mediation of the depressor response to afferent somatic nerve stimulation has not been clearly elucidated Attempts have been made in some previous investigations to determine whether the so called depressor area of the medulla oblongata constitutes the actual reflex centre but the evidence available from these studies is somewhat contradictory SCOTT (1925) found that local application of 1% strychnine nitrate in the region of the ala cinerea abolished the reflex fall in blood pressure due to vagal or somatic nerve stimulation while cauterization of this area affected the vagal response only In agreement with this latter observation LINDGREN and UVNAS (1954) found that elimination of the vagal depressor reflex by cauterization in the mediocaudal part of the rhomboid fossa left the somatic depressor response essentially unchanged The two different effects obtained by SCOTT might be due to a somewhat different localization of the bulbar relay stations or of the intrabulbar pathways for the vagal and the somatic depressor reflexes respectively Experiments which in fact indicate such a topographical differentiation of the bulbar depressor area will be presented below

To elucidate the relative importance of hindbrain structures as compared with propriospinal reflex connections for the mediation of the somatic depressor reflex two different types of experiments involving spinal and bulbar lesions respectively were *performed* in the present study

Results

a Effects of spinal cord lesions on the somatic depressor response

The first series of experiments to be described comprises 15 cats and is concerned with the location in the cervical spinal cord of pathways mediating

Comments

Stimulation of afferent spinal nerves with high stimulus strength and high impulse frequency can obviously produce a *widespread activation of the sympathetic vasoconstrictor fibres* but the discharge pattern was found to *differ considerably from the pressor reflex* adjustments produced by carotid occlusion The fact that the vessels of the skeletal muscles were relatively little engaged in the somatic pressor response — and that there might *occasionally even have been an active vasodilatation* in this region — may be important for the evaluation of the physiological significance of this circulatory reflex It is of interest in this connection that the defence alarm reaction which includes an activation of the *cholinergic vasodilator fibre system to the* skeletal muscles has been reflexly evoked in high decerebrate cats by various afferent influences among them nociceptive cutaneous stimuli (ABRAHAMS HILTON and ZBROZYNA 1960 a b) Stimulation of the diencephalic vasodilator area' produces besides the active muscle vasodilatation an intense activation of *the vasoconstrictor fibres to the vessels of the skin intestine* (FINDGREN 1955) and kidney (FEIGL JOHANSSON and LOFVING 1962 FEIGL and LOFVING 1962) and of the sympathetic fibres to the heart (ROSÉN 1961) The circulatory *response pattern induced from this hypothalamic integrative station of the* defence alarm reaction presents certain similarities to the circulatory adjustment produced by stimulation of the somatic pressor afferents which are probably nociceptive (see Chapter VI) It seems likely that these afferents evoke their circulatory effects by influencing cardiovascular centres at different levels of the central nervous system *A widespread vasoconstrictor response is* evidently mediated by reflex connections at the bulbospinal plane and this response pattern may dominate in anesthetized animals since the bulbar cardiovascular centres *are relatively more resistant to anesthetic agents* than are the higher levels of the central nervous system *It is known that a primitive* sort of defence behaviour occurs in bulbospinal preparations (KELLER 1932) but it is also clear that such reactions become more complex and purposeful in intact or decorticate animals The cardiovascular response pattern produced by *the somatic pressor afferents in the present experiments might represent* a kind of lower level framework for the circulatory adjustment associated with the defence behaviour This primitive pattern may normally be overcome by *the more differentiated diencephalic pattern* which includes an intense activation of the sympathetic vasodilator fibres to the skeletal muscles *If such a* reflex organization is assumed *the above mentioned occasional signs of active* muscle vasodilatation during a somatic pressor response may indicate that the diencephalic reflex centres were sometimes activated by the afferent nerve stimulations *in spite of the depressing* influence of anesthesia

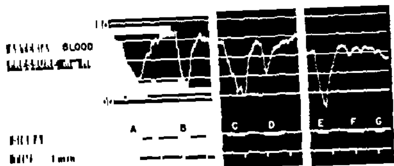




Fig 15 Cat 2 4 kg Chloralose Effects of acute ventral lesions in the upper cervical cord (C 1) on the depressor responses to afferent vagal and muscle nerve stimulation

A C and E Afferent stimulation of the left vagal nerve with 10 impulses/sec, 3 msec and 5 0 V

B D and F Afferent stimulation of the right hamstring nerve with 10 impulses sec, 1 5 msec and 3 0 V

C Afferent stimulation of the left hamstring nerve with 10 impulses sec, 1 5 msec and 3 0 V

The depressor response to afferent hamstring nerve stimulation is abolished by the ventral cord lesion while the vagal response is still obtained The ultimate size and localization of the lesion after the last dissection *between D and E,* is shown in sketch

The animal was atropinized curarized and kept under artificial respiration The vagal nerves were cut in the neck and one carotid artery was occluded

the pressor reactions obtained by high intensity stimulation of cutaneous nerves were also made

Fig 16 illustrates such an experiment in which depressor responses, elicited from the left hamstring nerve, and pressor responses, caused by afferent stimulation of the superficial peroneal nerve, were studied The effect on the blood pressure of carotid artery occlusion was used as a test for the intactness of the medullary centres and the descending vasomotor pathways One of the common carotid arteries was permanently occluded In A of Fig 16 the blood pressure response to afferent hamstring nerve stimulation is shown and B

the sympatho inhibitory influence of the group III afferents from muscle If the depressor responses to afferent muscle nerve stimulation could be abolished by interruption of some funiculus of the cervical cord without severing the descending bulbospinal tracts responsible for the tonic vasomotor fibre activity this would strongly suggest that supraspinal structures are essential for the mediation of the *depressor reflex from the peripheral muscle nerves*

Fig 15 illustrates a representative recording from one of the 7 experiments in which the ventral aspect of the cord at the level of C 1 was exposed for making lesions in the anterior fasciculi (p 24) A in this figure shows the depressor response obtained when the left vagal nerve was stimulated in the afferent direction Electrical stimulation of the central end of the right hamstring nerve (B) is seen to cause a decrease in arterial blood pressure of 50 mm Hg The ventral and ventrolateral fasciculi of the cord were then bilaterally severed by cautious dissections in several steps and the effects of hamstring and vagus nerve stimulation on the blood pressure were tested after each separate enlargement of the cord lesion The vasomotor response to the hamstring nerve stimulation gradually decreased (D) and finally a small extension of the lesion in the lateral directions abolished it almost completely (F) This could not be ascribed to a gradual impairment of the peripheral nerve itself since the contralateral hamstring nerve not previously used also failed to induce any significant depressor response at this state (G) The persistence of a good basal blood pressure and the undiminished depressor responses to vagal stimulation throughout the experiment (A C E) show that the descending vasomotor fibre systems were still functioning The final size and localization of the anatomical lesion as revealed by the serial sections are illustrated in the schematic drawing of the cord in Fig 15

Lesions within the anterior quadrants of the upper cervical cord have thus been shown to eliminate the depressor reflex induced from peripheral muscle nerves These results indicate that a spinal pathway subserving this cardio vascular reflex is localized in the ventral or ventrolateral fasciculi of the cord at this level and further that the reflex at least under the prevailing experimental conditions is not purely spinal but relayed over (or possibly conditioned from) supraspinal structures Unilateral lesions in the anterior part of the cord at C 1 failed to abolish the depressor response to hamstring nerve stimulation but seemed to reduce the response from the contralateral side more than that from the ipsilateral side

In order to control that the above mentioned elimination of the depressor reflex from muscle nerves was *not simply due to an unspecific traumatization* of the spinal cord lesions in the dorsal part of the cervical cord were produced in a set of 8 experiments Some observations on the effect of these lesions upon

hamstring nerve stimulation produced about the same reflex blood pressure fall as before (H) and occlusion of the carotid artery was still accompanied by a pronounced pressor response (J) In this as in other experiments with lesions involving the dorsal quadrants of the cervical cord the depressor effects of muscle nerve stimulation persisted unchanged provided the control blood pressure of the animals remained good

The experimental results obtained in the animals with such dorsal lesions are thus consistent with the view expressed above, that the fibre pathways subserving the somatic depressor reflex' are localized essentially in the anterior half of the cervical cord The effect of the cord lesions on the pressor response to afferent cutaneous nerve stimulation illustrated in Fig 16 will be considered below

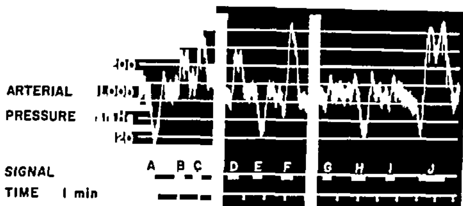
b Effects of experimental lesions in the medulla oblongata

A series of 10 experiments was performed to elucidate the role of the bulbar depressor area as a relay station for the depressor responses to afferent stimulation of muscle nerves These comprise the second type of experiments referred to on p 61

If the rhomboid fossa was exposed and an experimental lesion produced in the vicinity of the obex by means of dissections with fine forceps the depressor effect of afferent vagus nerve stimulation was markedly reduced abolished or even reversed to a slight rise in blood pressure The depressor response to electrical stimulation of the central end of a peripheral muscle nerve was on the other hand largely unchanged after such lesions which is in accordance with the results previously obtained by superficial cauterization of this bulbar area (SCOTT 1925 LINDGREN and UVNAS 1954) In Fig 17 the blood pressure falls induced by afferent stimulation of the hamstring nerves (A B) are seen to persist (E F) after dissections from the dorsal side in the mediocaudal region of the rhomboid fossa performed between C and D The initial vagal response (C) on the contrary was markedly reduced and the blood pressure returned towards its initial level in spite of continued stimulation (D) The lesions were now extended in the ventral direction so that a hole right thro[illegible]

[illegible] nerve stimulation also (G H) On the basis of this finding it was assumed that the bulbar relay station or the bulbar pathways subserving the somatic depressor reflex' might be constituted by nervous structures within central parts of the depressor area

To test this hypothesis experimental lesions were produced from the ventral



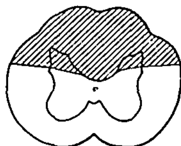


Fig 16 Cat 3 3 kg Chloralose Effect of dorsal cord lesions (C 3) on the blood pressure responses to afferent somatic nerve stimulation and to carotid artery occlusion

A, E and H Afferent stimulation of the right hamstring nerve with 10 impulses/sec 0 3 msec and 3 0 V

B, D, G and I Afferent stimulation of the left superficial peroneal nerve with 5 impulses/sec, 2 msec and 12 V

C, F and J Occlusion of the left common carotid artery (right one permanently occluded)

Note that the three different circulatory responses persist after transection of the dorsal columns between C and D The pressor response to cutaneous nerve stimulation is markedly reduced after dorsolateral extensions of the lesions between F and G (see sketch) while the effects of the hamstring nerve stimulation and the carotid occlusion are undiminished

The animal was atropinized, curarized and kept under artificial respiration The vagal nerves were cut in the neck

represents a pressor response obtained from the left superficial peroneal nerve C shows the effect of carotid artery occlusion After transection of the dorsal columns all three vasomotor responses were largely unchanged (D E F) while an extension of the lesions to involve the apical parts of the dorsal horns and the dorsolateral fasciculi on both sides (see drawing of the cord) caused a marked reduction in the pressor effect from the cutaneous nerve (G I) The

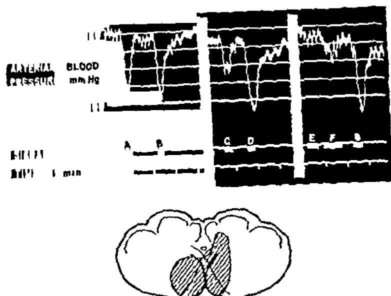


Fig 18 Cat 2 2 kg Chloralose Effects of experimental lesions in the ventral med o caudal part of the bulb on depressor responses to afferent vagal and *muscle nerve* stimulation

A C and E Afferent stimulation of the right hamstring nerve with 10 impulses/sec 1 msec and 3 V

B D and G Afferent stimulation of the left vagal nerve with 10 impulses sec, 3 msec and 5 V

F Afferent stimulation of left hamstring nerve with 10 impulses/sec 1 msec and 3 V

Experimental lesion in the ventromedial part of the bulb gradually enlarged between B and C and between D and E (see sketch of section from the upper level of the pyramidal decussation) Note that the response to hamstring nerve stimulation is progressively reduced while the vagal depressor effect persists

The animal was curarized and kept under artificial respiration The vagi were cut in the neck and one common carotid artery was occluded

The contralateral hamstring nerve not previously stimulated, evoked at this latest stage a *small blood* pressure fall only (F) The histological control showed the lesions to be localized at *the upper level* of the pyramidal decussation (see sketch in Fig 18) and a limited ventromedial intramedullary bleeding was traced 1—*2 mm above* and below the mechanical destruction The lesion was thus in this animal not strictly *confined to* the ventral portions of the depressor area but results having the same main characteristics were obtained in *other* experiments where the lesions were *placed* at a more rostral level

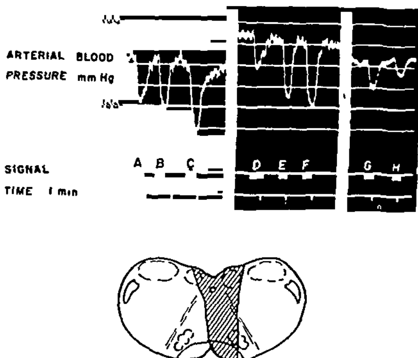


Fig 17 Cat 2 3 kg Chloralose Effects of experimental lesions in the medial part of the medulla oblongata on depressor responses to afferent vagal and muscle nerve stimulation
A, E and H Afferent stimulation of the left hamstring nerve with 10 impulses sec, 0 5 msec and 2 V
B, F and G Afferent stimulation of the right hamstring nerve with 10 impulses/sec, 0 5 msec and 2 V
C and D Afferent stimulation of the vagal nerve with 10 impulses/sec, 3 msec and 5 V.
Experimental lesion produced in the region of the obex between C and D and extended into the ventral parts of the medulla between F and G (see sketch of section just below *the obex)*

Note that the vagal depressor response is considerably reduced by the dorsal lesion, which does not influence the effect of hamstring nerve stimulation The latter response is reduced after the lesion encroaches upon ventral structures

The animal was curarized and kept under artificial respiration The vagi were cut in the neck and one common carotid artery was occluded

aspect of the bulb (p 25) and the effects on the blood pressure of afferent vagal and muscle nerve stimulation were studied before and after such lesions *Fig 18 illustrates one of these experiments* where marked blood pressure falls could be obtained both from the right hamstring and the left vagus nerves (A and B respectively) Dissections in the ventral, mediocaudal part of the bulb caused a gradual impairment of the hamstring response (C and E) while the vagal depressor effects were not affected to any detectable extent (D, G)

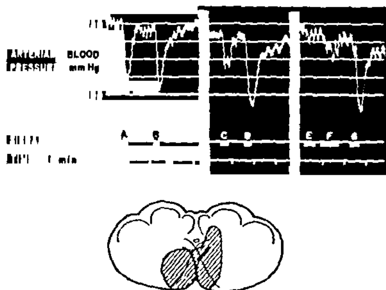


Fig 18 Cat 2 2 kg Chloralose Effects of experimental lesions in the ventral medio-caudal part of the bulb on depressor responses to afferent vagal and muscle nerve stimulation

A C and E Afferent stimulation of the right hamstring nerve with 10 impulses sec 1 msec and 3 V

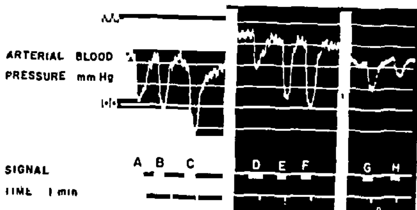
B D and G Afferent stimulation of the left vagal nerve with 10 impulses sec, 3 msec and 5 V

F Afferent stimulation of left hamstring nerve with 10 impulses sec 1 msec and 3 V

Experimental lesion in the ventromedial part of the bulb gradually enlarged between B and C and between D and E (see sketch of section from the upper level of the pyramidal decussation) Note that the response to hamstring nerve stimulation is progressively reduced while the vagal depressor effect persists

The animal was curarized and kept under artificial respiration The vagi were cut in the neck and one common carotid artery was occluded

The contralateral hamstring nerve not previously stimulated, evoked at this latest stage a small blood pressure fall only (F) The histological control showed the lesions to be localized at the upper level of the pyramidal decussation (see sketch in Fig 18) and a limited ventromedial intramedullary bleeding was traced 1—2 mm above and below the mechanical destruction The lesion was thus in this animal not strictly confined to the ventral portions of the depressor area but results having the same main characteristics were obtained in other experiments where the lesions were placed at a more rostral level



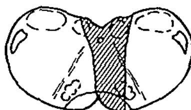


Fig 17 Cat 2 3 kg Chloralose Effects of experimental lesions in the medial part of the medulla oblongata on depressor responses to afferent vagal and muscle nerve stimulation

A, E and H Afferent stimulation of the left hamstring nerve with 10 impulses sec, 0 5 msec and 2 V

B, F and G Afferent stimulation of the right hamstring nerve with 10 impulses/sec, 0 5 msec and 2 V

C and D Afferent stimulation of the vagal nerve with 10 impulses/sec, 3 msec and 5 V

Experimental lesion produced in the region of the obex between C and D and extended into the ventral parts of the medulla between F and G (see sketch of section just below the obex)

Note that the vagal depressor response is considerably reduced by the dorsal lesion which does not influence the effect of hamstring nerve stimulation The latter response is reduced after the lesion encroaches upon ventral structures

The animal was curarized and kept under artificial respiration The vagi were cut in the neck and one common carotid artery was occluded

aspect of the bulb (p 25) and the effects on the blood pressure of afferent vagal and muscle nerve stimulation were studied *before and after such lesions* Fig 18 illustrates one of these experiments where marked blood pressure falls could be obtained both from the *right hamstring* and the *left vagus nerves* (A and B respectively) Dissections in the ventral, mediocaudal part of the bulb caused a gradual impairment of the hamstring response (C and E) while the vagal depressor effects were not affected to any detectable extent (D G)

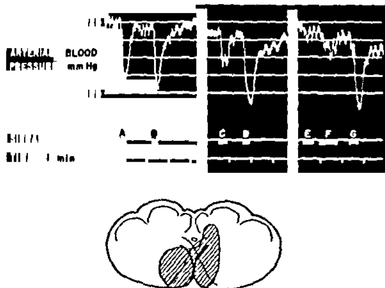


Fig 18 Cat " 2 kg Chloralose Effects of experimental lesions in the ventral medio caudal part of the bulb on depressor responses to afferent vagal and muscle nerve stimulation.

A C and E Afferent stimulation of the right hamstring nerve with 10 impulses sec 1 msec and 3 V

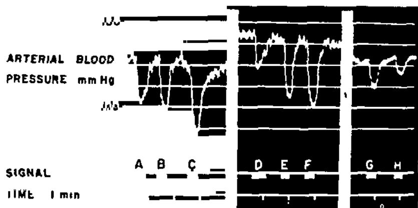
B D and G Afferent stimulation of the left vagal nerve with 10 impulses sec 3 msec and 5 V

F Afferent stimulation of left hamstring nerve with 10 impulses sec 1 msec and 3 V

Experimental lesion in the ventromedial part of the bulb gradually enlarged between B and C and between D and E (see sketch of section from the upper level of the pyramidal decussation) Note that the response to hamstring nerve stimulation is progressively reduced while the vagal depressor effect persists

The animal was curarized and kept under artificial respiration The vagi were cut in the neck and one common carotid artery was occluded

The contralateral hamstring nerve not previously stimulated evoked at this latest stage a small blood pressure fall only (F) The histological control showed the lesions to be localized at the upper level of the pyramidal decussation (see sketch in *Fig 18*) and a limited ventromedial intramedullary bleeding was traced 1–2 mm above and below the mechanical destruction The lesion was thus in this animal not strictly confined to the ventral portions of the depressor area but results having the same main characteristics were obtained in other experiments where the lesions were placed at a more rostral level



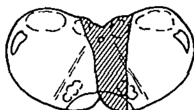


Fig 17 Cat 2 3 kg Chloralose Effects of experimental lesions in the medial part of the medulla oblongata on depressor responses to afferent vagal and muscle nerve stimulation
A, E and H Afferent stimulation of the left hamstring nerve with 10 impulses/sec, 0 5 msec and 2 V
B, F and G Afferent stimulation of the right hamstring nerve with 10 impulses/sec, 0 5 msec and 2 V
C and D Afferent stimulation of the vagal nerve with 10 impulses/sec, 3 msec and 5 V
Experimental lesion produced in the region of the obex between C and D and extended into the ventral parts of the medulla between F and G (see sketch of section just below the obex)

Note that the vagal depressor response is considerably reduced by the dorsal lesion which does not influence the effect of hamstring nerve stimulation The latter response is reduced after the lesion encroaches upon ventral structures

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nerve with the same impulse characteristics as before failed to produce any blood pressure fall whatsoever (D) and the contralateral hamstring nerve was as ineffective in that respect (E) On the other hand the depressor response to afferent vagal nerve stimulation was seen to persist unchanged (F) G and H in Fig 19 show that pressor responses could still be elicited by high intensity — high frequency stimulation of the hamstring nerve and by occlusion of the remaining carotid artery

The results of the experiments presented above indicate when taken together that the vagal depressor reflex is mediated via the dorsal part of the bulbar depressor area while nervous structures with a more ventral localization seem to be engaged in the depressor reflex originating from muscle afferents Higher levels of the brain stem do not seem to be essential for this latter reflex since it could be demonstrated in the acute decerebrate animals (see p 35) Furthermore in one of the present experiments on a chloralose anesthetized cat extensive lesions within the ventral half of the brain stem at the level of the ponto medullary transition did not affect the depressor responses induced from muscle nerves

c Effects of spinal cord lesions on the somatic pressor response

Some observations made on the effect of different cervical cord lesions upon the pressor responses to high intensity stimulation of afferent somatic nerves will be briefly accounted for before the experimental findings presented in this chapter are more thoroughly discussed In Fig 16 the pressor response induced from a cutaneous nerve was seen to be markedly reduced after transection of the dorsal parts of the lateral funiculi (G I) but this was by no means a constant finding in these experiments Fig 20 illustrates the results from another animal in which dorsal cord lesions were made at the level of C3 A and B represent pressor responses induced by high intensity stimulation of the left superficial peroneal nerve at the beginning of the experiment The blood pressure rise was still obtained after the dorsal columns had been almost completely transected (C D) and a bilateral enlargement of the lesion into the dorsolateral fasciculi including the zone of Lissauer did not interfere to any greater extent with the pressor response (E F) It may also be pointed out that ventral cord lesions like that depicted in Fig 15 were never seen to reduce significantly the reflex blood pressure rises induced from peripheral nerves The experimental results were thus less conclusive as regards the localization of the spinal pathways for fibres related to the pressor responses Some of the experiments (*e g* Fig 16) were largely in agreement with those of Ranson and von Hess (1915) and Ranson (1916)

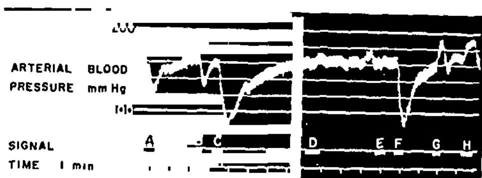




Fig 19 Cat 2 6 kg Chloralose Effects of ventromedial lesions in medulla oblongata on blood pressure responses to afferent vagal and muscle nerve stimulation

A, B and D Afferent stimulation of the right hamstring nerve with 10 impulses/sec 0 5 msec and 3 V

C and F Afferent stimulation of the left vagal nerve with 10 impulses/sec, 3 msec and 5 V

E and G Afferent stimulation of the left hamstring nerve in E with 10 impulses/sec, 0 5 msec and 3 V and in G with 50 impulses/sec, 3 msec and 10 V

H Occlusion of one of the common carotid arteries the other one being permanently occluded throughout the experiment

Note that the depressor response to afferent muscle nerve stimulation is abolished by the lesion in the ventromedial part of the medulla oblongata The lesion is localized at a higher level than in Fig 18 Sketch of section about 1 mm above the obex

The animal was atropinized, curarized and kept under artificial respiration and the vagi were cut in the neck

within the ventral parts of the medial reticular formation One such experiment is illustrated in Fig 19 The depressor responses obtained from the right hamstring nerve (A and B) amounted to about 30 mm Hg in this animal Afferent stimulation of the left vagal nerve produced a pronounced fall in blood pressure as demonstrated in C A mechanical lesion was made in the ventromedial region of the medulla and this was gradually enlarged to the size shown in the sketch of Fig 19 The extent of the anatomical lesion in the rostrocaudal plane was about 1 5 mm Stimulation of the right hamstring

vasomotor system is still incomplete Little is known for example about the exact localization within the spinal cord of the descending fibre systems which are responsible for the bulbar control of the thoraco lumbar vasoconstrictor neurones According to the investigations by CHEN *et al* (1937 b 1938) and by WANG and RANSON (1939 b) pressor effects of bulbar or hypothalamic origin are mediated *via* efferent pathways in the ventrolateral funiculi of the spinal cord while descending sympatho-inhibitory pathways are said to be localized in the dorsolateral section of the cord (LIM WANG and YI 1938) ALEXANDER (1946) adopted the view that the peripheral vasoconstrictor fibre activity is normally determined by the balance between these excitatory and inhibitory autonomic bulbospinal systems which he considered to emerge from the bulbar pressor and depressor areas respectively There is however, little experimental support for this concept of the anatomical and functional arrangement of the vasomotor control (cf UVNAS 1960 b)

In the experiments with spinal cord lesions presented above the functional integrity of the descending vasomotor fibre systems was regularly controlled by observing the blood pressure responses to afferent vagal stimulation and/or carotid artery occlusion Therefore the abolition of the depressor response to activation of muscle afferents by experimental lesions in the anterior part of the upper cervical cord must be ascribed to an interruption of some pathways selectively engaged in this somatic depressor reflex but not essential for the vagal or carotid sinus reflexes It is reasonable to assume that these pathways correspond to ascending fibre systems by which the primary group III fibres exert their sympatho-inhibitory influence upon the bulbar cardiovascular centres That they might constitute instead a descending conditioning system facilitating the impulse transmission in propriospinal reflex connections between group III afferents and preganglionic vasoconstrictor neurones seems less likely for the following reasons Firstly, the fact that a vagal bradycardia is part of the depressor response pattern shows that the afferent impulses influence circulatory centres at the bulbar level Secondly a definite 'local sign has not been observed in the reflex vascular response Thirdly preliminary experiments (JOHANSSON 1961) have suggested that ventrolateral lesions in the *lower* segments of the cervical cord interfere with the depressor reflex induced from the hamstring nerves without affecting the corresponding response to stimulation of forelimb afferents entering the cord above the lesions

In view of the increasing body of experimental evidence *for the existence* of descending fibre systems mediating a supraspinal control of synaptic transmission at the lower levels of the neuraxis (see for example HOLMQUIST and LUNDBERG 1961) definite conclusions on questions of this kind are difficult to draw from experiments with spinal cord lesions It is very possible

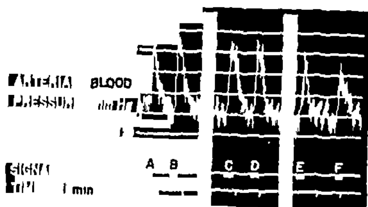


Fig 20 Cat 2 3 kg Chloralose Effects of dorsal cord lesions at the level of C 3 on pressor responses to afferent stimulation of the left superficial peroneal nerve with 12 impulses/sec 2 msec and 10 V (A–F)

Note that the pressor responses are undiminished after section of the dorsal columns (between B and C) and that they are also little affected by lesions in the dorsolateral fasciculi (produced between D and E)

according to which the ascending pressor path is represented in the dorsolateral tracts of Lissauer while other experiments (e g Fig 20) indicated that the afferent fibres if exerting all their vasomotor influence on supraspinal levels have also other projections in the cord These divergencies in the results concerning the pressor responses could not be related to any definite characteristics of the dorsal cord lesions as revealed by the histological examinations

Comments

The importance of nervous structures in the medulla oblongata for the normal cardiovascular control is well established but the available information concerning the functional and anatomical organization of the bulbospinal

According to ALEXANDER (1946) it is a tonically active sympatho-inhibitory centre and a reflex station for a variety of depressor responses exerting its influence on spinal autonomic neurones via separate descending pathways. On the other hand LINDGREN and UVNÄS (1954) suggested that the medio-caudal part of the rhomboid fossa should not be looked upon as a reflex centre but merely as an anatomical region where afferent depressor fibres affecting the tonically active vasomotor centre happen to be concentrated. In any case sympatho-inhibitory influences from various sources converge towards this medial part of the bulbar reticular formation as demonstrated in a recent study by LOFVING (1961 a). He found that the inhibitory effects upon the tonic vasoconstrictor fibre activity exerted by the rostral cingulate gyrus and by vagal and carotid sinus afferents were all mediated via this bulbar area. The present experiments suggest that the sympatho-inhibitory effect of somatic group III afferents is also relayed over the depressor area of the medulla oblongata though rather by way of its more ventral section. Without going into semantics it seems reasonable that such a relatively circumscribed region of the central nervous system deserves to be called a cardiovascular centre as it has so many afferent connections both from higher levels of the central nervous system from cardiovascular proprioceptors and from somatic afferent fibre systems all inducing the same pattern of circulatory response. Whether it is a centre also in the sense that it exhibits spontaneous intrinsic activity independent of afferent inflow is at present less certain (OBERHOLZER 1960). The differentiation of the depressor area into a dorsal and a ventral subdivision associated with vagal and somatic depressor responses suggested by the present experiments seems to be consistent with what is known from neuroanatomical and neurophysiological investigations concerning the intrabulbar pathways and terminations of vagal and spinoreticular afferent fibres within the caudal part of the medulla (INGRAM and DAWKINS 1945, ANDERSON and BERRY 1956, 1959, NAUTA and KUYPERS 1957, ROSSI and BRODAL 1957).

Both the experiments with ventral cord lesions and those with lesions in the medullary depressor area indicate that hindbrain mechanisms are at least under the prevailing experimental conditions of relatively greater importance for the somatic depressor reflex than possible propriospinal connections between the afferent and efferent systems. In investigations concerned with cardiovascular responses in chronic spinal animals and in spinal man there are numerous observations of vasoconstrictor responses to various afferent stimuli (e.g. BROOKS 1933, 1935, SAHS and FULTON 1940, WHITTERIDGE 1960). On the other hand the possibilities for reflex vasodilatation by inhibition of tonic vasoconstrictor activity in the spinal state has

that such mechanisms are engaged also in the visceromotor control It is however, of interest in this connection that investigations with other techniques have demonstrated ascending pathways in the ventral parts of the cord related to thin myelinated afferents of various origin Thus for example COLLINS and RANDT (1956) found that stimulation of this type of afferent fibres in the sciatic and superficial radial nerves evoked potentials in the antero lateral region of the cord at the levels of T 11 and C 2 respectively The recent study by COLLINS and RANDT (1961) on the relationship between afferent fibre characteristics and functional organization of ascending spinal tracts is also of some relevance to this question Ventral spinal pathways have been described for the A gamma delta fraction of the splanchnic nerves (AMASSIAN 1951 AIDAR GEOHEGAN and UNGEWITTER 1952, DOWNMAN and EVANS 1957) In a study of the effect of various cord lesions upon the pupillomotor responses produced by stimulation of cutaneous A delta and C fibres EVANS (1961) concluded that both fibre systems project diffusely into the lateral funiculi although there was a tendency for the ascending fibres related to the former group to lie more ventrally LUNDBERG and OSCARSSON (1962) described two ventral pathways which are activated by the flexor reflex afferents to which the muscular group III fibres belong but such pathways have been found also in the dorsolateral fasciculi (HOLMQUIST, LUNDBERG and OSCARSSON 1959) The localization of the experimental lesions in the anterior part of the cervical cord at C 1 which were found to abolish the depressor response to afferent muscle nerve stimulation (Fig 15 above) corresponds well with the representation of spinobulbar fibre tracts as revealed in neuro anatomical studies of fibre degeneration (e g ROSSI and BRODAL 1957 ANDERSON and BERRY 1959)

The experiments with lesions in the medulla oblongata indicate that there is a dorso ventral topographical differentiation of the bulbar depressor area as regards the mediation of sympatho inhibitory effects from the vagal and the peripheral muscle nerves respectively As the sympatho inhibitory effect of peripheral muscle afferents was found in this study to be dependent upon structures in the ventrocaudal part of the bulb it is not surprising that superficial cauterizations from the dorsal side in the distal part of the rhomboid fossa (SCOTT 1925 LINDGREN and UVNAS 1954) failed to interfere with this vasomotor reflex The findings presented above should however not be taken as evidence for the existence of two distinctly separated reflex centres subserving the vagal and the 'somatic' depressor reflex respectively for they may merely reflect an anatomical differentiation within a functionally fairly homogeneous centre

The true nature of the bulbar depressor area is still a matter of dispute

CHAPTER VI

General conclusions and comments

The aim of the present investigation was to study some characteristics of the cardiovascular reflex responses which can be elicited by activation of afferent somatic fibre systems Most of the interest has been focussed upon the depressor effects obtained by stimulation of muscle nerves The present chapter will be devoted to a short review of the previous chapters and to a tentative discussion of the functional significance of the reflexes studied

It was pointed out in *Chapter I* that the currently available information concerning the response patterns of many reflex circulatory adjustments is incomplete due to the fact that *regional* vascular reactions have not been sufficiently studied In view of some recent investigations with closer analyses of the cardiovascular effects associated with depressor responses to stimulation of cortico hypothalamic structures and to activation of vagal or carotid sinus afferents (FOLKOW JOHANSSON and ÖBERG 1959, LOFVING 1961 a, b), t was considered of importance to study also the response pattern of the depressor reflex obtained by afferent stimulation of somatic sensory nerves The previous literature with bearing on the present problems was surveyed

Simultaneous recordings of arterial blood pressure and regional blood flows in different tissues were used for studying the circulatory response patterns The reflexly induced changes in the discharge rate of the vasoconstrictor fibres to the various regions can be approximately evaluated from observations of changes in local vascular resistance since the frequency response curves of the resistance vessels of the different regions are known from earlier investigations (CELANDER and FOLKOW 1953, CELANDER 1954), and can further be checked in the individual experiment The advantages of this methodological approach as compared with direct electrophysiological recordings of vasoconstrictor fibre activity were discussed in *Chapter II* In order to elucidate the importance of different spinal pathways and of certain nervous structures in the medulla oblongata for the mediation of the pressor and depressor responses to afferent somatic nerve stimulations, these circulatory reflexes were studied in animals subjected to various acute lesions in the spinal cord and in the bulb The possibilities and difficulties associated with functional

attracted less attention In spinal monkeys FULTON (1938) described a reflex vasodilatation in the hind leg induced by warming of the contralateral limb and KUNTZ (1945) saw intestinal vasoconstriction and vasodilatation in spinal rats as responses to thermal stimulation of the skin It is quite possible that the group III afferents from muscle make reflex connections with the spinal sympathetic vasoconstrictor neurones and that they exert a sympatho inhibitory influence also in the chronic spinal preparation but experimental studies with bearing on this question seem to be lacking If such reflex connections exist at the spinal level the present experiments indicate that they are 'normally' of little functional importance

The present experimental results concerning the spinal pathways for the pressor responses induced from cutaneous nerves will only be briefly commented upon The studies by RANSON and VON HESS (1915) referred to above, indicated that the ascending spinal path for this 'somatic' pressor reflex is confined to the 'tracts of Lissauer', and according to RANSON (1916) the reflex is mediated *via* supraspinal cardiovascular centres CHEN *et al* (1937 a) arrived at similar conclusions Experiments in this series (Fig 20) have indicated that the dorsolateral fasciculi are not the only spinal pathways by which cutaneous afferents induce pressor effects even if experimental lesions of these tracts were sometimes seen to reduce markedly this vasomotor response (Fig 16) As judged from the investigation by LAPORTE and MONTASTRUC (1957) the pressor responses obtained by low frequency stimulation of cutaneous nerves are mainly due to C fibre activation but thin medullated afferents might also contribute to the responses The persistence of these pressor effects after dorsolateral cord lesions in some of the present experiments is in agreement with the results of EVANS (1961) concerning the spinal pathways for thin medullated and non medullated cutaneous afferents A mediation of the excitatory influence on the thoraco lumbar vasoconstrictor neurones *via* purely spinal reflex arcs cannot be excluded here (cf RANSON 1916) As mentioned above such neuronal connections are known to be active in the chronic spinal animal and they might perhaps be of functional significance also in the animal with an intact brain if the excitability of the preganglionic vasomotor neurones is high (cf ADAMS RAY and NORLÉN 1951) Further experimental investigations seem to be needed before the organization of this 'somatic pressor reflex' can be definitely understood It is very probable that the afferent fibres responsible for it exert their action on cardiovascular structures at many different levels of the central nervous system

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identifications of spinal pathways on the basis of experimental cord lesions were also considered in *Chapter II* Material anesthesia operative procedures stimulating and recording techniques etc were described

Chapter III was devoted to a description of the blood pressure changes obtained when various afferent somatic nerves were stimulated with different stimulus strengths and impulse frequencies Pronounced depressor effects were induced by activation of muscle afferents especially if the buffering capacity of the baroreceptors had been reduced or eliminated Determinations of the stimulus threshold of the depressor afferents in muscle nerves as compared with that of the alpha efferents or of the group I afferents indicated that the blood pressure falls were mainly due to activation of group III fibres The earlier observations that blood pressure falls are predominantly obtained with low impulse frequencies were confirmed but a reversal of the response was not seen even if the group III fibres were stimulated at high impulse rates (100—400/sec) Pressor effects were obtained from muscle nerves if the stimulus strength was sufficiently raised to activate also the non medullated afferents The impulse frequency had then to be increased too since at a concomitant stimulation of group III and C fibres with low frequencies the depressor influence of the former was generally predominant In experiments with adequate stimulation squeezing or pinching of the muscles was seen to evoke the most pronounced depressor responses

Stimulation of cutaneous nerves was found to produce mainly pressor effects and only slight blood pressure falls were seen at low stimulus frequencies and moderate intensities The depressor afferents in cutaneous nerves seemed to have about the same threshold characteristics as those of the muscle nerves

Experimental results obtained in decerebrate animals did not differ significantly from those of the anesthetized group

The cardiovascular response pattern of the depressor reflex associated with activation of group III afferents was analysed with regard to changes in regional vascular resistance and in heart rate in the first section of *Chapter II* Recordings of venous outflow from muscle kidney intestine and skin showed that a reflex vasodilatation was induced in all these vascular beds by the afferent muscle nerve stimulation That these effects were not due to a reflex activation of cholinergic vasodilator fibres or to changes in the adrenomedullary catechol secretion was evident from the fact that atropinization and denervation of the adrenal glands had no significant influence upon the experimental results The induced vasodilatations could therefore be ascribed solely to an inhibition of the impulse activity in the sympathetic vasoconstrictor fibres supplied to the different vascular regions This reflex inhibition of tonic sympathetic activity included the constrictor fibres to

capacitance vessels and the accelerans fibres to the heart and was further combined with a vagal bradycardia The reflex vasodilatation induced from the group III fibres was most pronounced within the skeletal muscle region due to the fact that in this area the prevailing neurogenic vascular tone is relatively high, especially in a state of reduced baroreceptor and/or increased chemoreceptor activity The inhibitory influence of the group III afferents on the renal vasoconstrictor neurone pool could be demonstrated after a tonic activity had been 'built up' in the sympathetic fibres to the kidney A 'somatic depressor response was accompanied by a rather small vasodilatation in the skin even if a high neurogenic tone was present in the cutaneous vessels It was suggested therefore that this vascular bed is predominantly controlled by thermoregulatory mechanisms

The response pattern of the somatic depressor reflex' was discussed with regard to its general hemodynamic consequences

Some observations on the regional vascular responses associated with the *somatic pressor reflex induced from cutaneous or mixed somatic nerves* were presented in the second section of Chapter IV This circulatory reflex involves an increase in vasoconstrictor fibre discharge, but the response pattern does not coincide with that obtained when the baroreceptors are unloaded or the chemoreceptors activated (cf LOFVING 1961 a b) The renal blood flow resistance was for instance found to increase to a relatively great extent as compared with the slight or insignificant vascular reactions produced in this region by changes of the baroreceptor or chemoreceptor activity The opposite may be said about the vessels of the skeletal muscles

The possible functional relationship between the 'somatic pressor reflex' and the cardiovascular adjustments associated with the 'defence reaction was discussed

The aim of the experiments presented in *Chapter V* was to elucidate the relative importance of bulbar and spinal reflex connections for the mediation of the depressor response to activation of group III afferents This depressor reflex was selectively abolished by experimental lesions in the ventral quadrants of the upper cervical cord a procedure which did not interfere with vagal or carotid sinus reflex responses These findings indicate that the group III afferents exert their sympatho inhibitory influence on supraspinal cardio vascular centres *via* ascending pathways in the anterior parts of the cord The effects of localized lesions within the bulbar depressor area on blood pressure responses to afferent vagal and muscle nerve stimulation were studied in a separate series of experiments The vagal depressor response was abol [illegible]
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The pressor effects obtained by high intensity stimulation of cutaneous nerves were sometimes seen to be markedly reduced by cervical cord lesions in the dorsal parts of the lateral funiculi but this was not a constant finding It was suggested that ascending pathways related to the primary pressor afferents may exist also in other sections of the cord or that propriospinal reflex connections to the preganglionic sympathetic neurones known to be of importance in the spinal state may be functionally active also in the intact organism

The functional significance of the cardiovascular responses to stimulation of sensory nerve fibres may be considered with regard to the hemodynamic consequences of the reflex adjustments and with regard to the present state of knowledge concerning the nature of the responsible afferent fibre systems

The fact that activation of muscle afferents can produce a reflex vasodilatation which is generally though not always most pronounced in the muscle tissue might at first sight suggest that these fibres represent a mechanism for securing the blood supply preferentially to the skeletal muscles for example during phasic or tonic contraction This aspect of the actual vasomotor response was discussed by SKOGLUND (1960) in connection with his observations of the reflex circulatory adjustments initiated by passive stretch of muscles and by artificial stimulation of muscle afferents procedures which generally produced a decreased flow resistance in the muscles

The present investigation has shown that the thin medullated muscle afferents produce a generalized depression of the cardiovascular equilibrium This is accomplished by activating vagal cardio inhibitory fibres and by depressing the tonic activity in the accelerans fibres to the heart and in the vasoconstrictor fibres to resistance and capacitance vessels It is true that the blood flow resistance is often reduced to a relatively greater extent in the skeletal muscle area but this is simply a reflection of the fact that the prevailing vasoconstrictor tone is generally more pronounced in this region Actually a depressor response like that induced by afferent stimulation of muscle nerves is not suited for securing an increased blood supply to activated skeletal muscles for the following reasons During active contraction local metabolites will promptly initiate the regional vasodilatation required If this was combined with a widespread decrease of flow resistance venous pooling of blood and reduction of heart activity this reflex sympatho inhibitory effect would rather tend to *decrease* the muscle blood flow by

reducing the pressure head over the region The cardiovascular system is more appropriately adjusted for improving the blood supply to the skeletal muscles by the opposite type of neurogenic response viz a widespread sympathetic activation which is known to take place in muscular work This may further be exemplified by the circulatory changes accompanying the 'defence reaction' (ABRAHAMS HILTON and ZBROZYNA 1960 a b) where blood 'depots' are emptied muscle vessels actively dilated flow resistance in other circuits increased cardiac output raised and the arterial pressure maintained or augmented

As discussed in Chapter IV a massive activation of the group III afferents in the intact organism can rather be supposed to cause an acute circulatory collapse especially if the buffering capacity of the baroreceptors is previously reduced as in situations where blood loss or hydrostatic pooling of blood has led to a reduction in the arterial mean and pulse pressures The cardiovascular adjustment induced by the muscle afferents when developed to its full extent displays in fact hemodynamic characteristics analogous to those seen during a vaso vagal syncope' in man A very similar, probably identical circulatory reaction was elicited from an area in the anterior hypothalamus (FOLKOW, JOHANSSON and ÖBERG 1959) from the rostral cingulate gyrus and from vagal and carotid sinus afferents (LOFVING 1961 a) These different sympatho inhibitory mechanisms including the somatic depressor reflex' all seem to exert their cardiovascular influence *via* the bulbar depressor area

The afferent fibres responsible for the 'somatic depressor responses' seem to be present in the largest proportion in muscle nerves and they are mainly confined to the group III the vague indications that group II fibres might contribute to the sympatho inhibitory effect may be explained simply by a threshold overlap of fibres functionally more related to the group III (cf PAINTAL 1960) As judged from the slight blood pressure falls obtained from cutaneous nerves these contain relatively few depressor afferents which are probably thin medullated fibres Whether these are in any way functionally related to the group III afferents from muscles is not known It does not seem entirely improbable that they also emanate from such deeper tissues in the peripheral parts of the limbs as fasciae tendons ligaments etc whose afferent fibres may join the regional cutaneous nerves

The somatic depressor response should be considered also in relation to the presently available information concerning the natural mode of activation of the thin medullated afferents especially those from muscle BESSOU and LAPORTE (1960 1961) made an analysis of the group III fibres in the soleus and gastrocnemius muscles of the cat They found that these fibres constitute a somewhat heterogeneous group with regard to the size and localization of the receptive fields and the rate of adaptation Passive stretch and contraction of

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and even if these have been shown to mediate also other sensory modalities than pain (see e g DOUGLAS and RITCHIE 1962) there seems to be no reason to revise the concept of this pressor reflex as being part of a nociceptor response pattern

The concept of pain as a sensory experience can probably be adequately defined only on the basis of its psychological effects and conclusions concerning the physiology of this sensation based on its behavioural or autonomic manifestations in experimental animals must therefore always be to some extent conjectural and speculative An animal s *perception* of pain cannot be recorded or estimated but only its *reactions* to such stimuli which by reference to human experience can be expected to be painful Even if the feeling of discomfort and uneasiness is the characteristic of all types of human pain there are certain apparent differences in the quality of the sensation and in the reactions that it evokes The site of origin of the pain is of great importance in this respect which may be illustrated by the following quotation taken from WOLFF and WOLF (1958 pp 23—24) In general cutaneous pain differs from visceral pain in possessing a bright quality which seems to exert an exhilarating action and commonly incites the individual affected to fight or flight This pattern has biologic usefulness since assaults from a hostile environment are likely first to strike the skin Visceral pain on the other hand is characterized by a deeper aching quality which seems to exert a depressing effect is commonly associated with nausea and followed by inactivity This pattern too appears to have biological significance since fight or flight would be fruitless against assaults from within

It may be possible to distinguish between two different types of pain also with regard to the kinds of stimuli which evoke the sensation Thus the one type is produced mainly by mechanical deformation of the tissues *i e* by pressure stretch distension etc while the other is more obviously connected with tissue damage which is usually looked upon as the adequate stimulus for the nociceptive nerve terminals The former type seems to be predominantly represented in deep structures For example distension of a hollow viscus is often associated with pain while the same organ may be cut or burnt without giving rise to any sensation of that kind This effect of mechanical deformation would correspond to the aching depressing nauseating deep pain referred to above

Some of the reactions to pain and especially those mediated by the autonomic nervous system are obviously independent of the perception of the pain since they can be observed in the unconscious individual for example during surgical anesthesia or even in a decerebrate animal These response patterns are consequently not secondary to the psychological phenomenon but can be

the muscles were not very effective in activating any of these fibres but most of them were activated by pressure PAINTAL (1960) also made a functional analysis of group III muscle afferents and arrived at similar conclusions He suggested that these fibres may mediate the sensation of muscle pain produced by squeezing the muscle or by injecting hypertonic salt solutions intramuscularly, and he introduced the term 'pressure pain receptors' to denote this According to PAINTAL the group III fibres are not activated by asphyxia and are therefore probably not responsible for the pain of muscle ischemia C-fibres from muscle seem to be related to this latter type of sensation (BESSOU and LAPORTE 1958)

If the afferents responsible for the pronounced depressor reactions described above, were tonically active or activated in ordinary muscle work this would seem to jeopardize seriously the nervous control of the circulation The idea that the group III fibres or at least some of them, should be nociceptive is therefore of great interest for the evaluation of their cardiovascular effects In those of the present experiments where adequate stimuli were used (p 33) firm pressure on the muscles was found to be the most effective method for producing a reflex blood pressure fall (cf VINCENT and THOMPSON 1928) which is thus in accordance with the above mentioned results of PAINTAL (1960) and BESSOU and LAPORTE (1960 1961) Attention may also be drawn to the fact that similar or identical cardiovascular depressor effects — characterized by a blood pressure fall a vagal bradycardia and a venous and arteriolar dilatation — could be elicited in man during abdominal surgery by distension pulling pinching etc of the internal organs procedures which are known to be painful (FOLKOW *et al* 1962) These reflex circulatory phenomena could be prevented by splanchnic blockade and they could on the other hand be reproduced by electrical stimulation of splanchnic nerve branches A rough determination of the threshold intensities required for activation of these visceral afferents indicated that they were thin medullated fibres The threshold characteristics the natural mode of activation and the reflex circulatory effects of these visceral afferents are so closely similar to those of the group III fibres from skeletal muscles that it is tempting to draw a functional parallel between the two groups of fibres which both emanate from deep lying tissues (see further below)

In contrast the majority of the somatic afferent fibres responsible for the *pressor* response appear to emanate from the body surface The somatic pressor response has been looked upon as a nociceptive autonomic reflex and it has actually been used as an objective sign of pain in animal experiments concerned with the physiology of sensations The more pronounced blood pressure rises obtained from somatic nerves are due to activation of C fibres

and even if these have been shown to mediate also other sensory modalities than pain (see *e g* DOUGLAS and RITCHIE 1962) there seems to be no reason to revise the concept of this pressor reflex as being part of a nociceptor response pattern

The concept of pain as a sensory experience can probably be adequately defined only on the basis of its psychological effects and conclusions concerning the physiology of this sensation based on its behavioural or autonomic manifestations in experimental animals must, therefore, always be to some extent conjectural and speculative An animal s *perception* of pain cannot be recorded or estimated but only its *reactions* to such stimuli which by reference to human experience, can be expected to be painful Even if the feeling of discomfort and uneasiness is the characteristic of all types of human pain there are certain apparent differences in the quality of the sensation and in the reactions that it evokes The site of origin of the pain is of great importance in this respect which may be illustrated by the following quotation taken from WOLFF and WOLF (1958 pp 23—24) 'In general cutaneous pain differs from visceral pain in possessing a bright quality which seems to exert an exhilarating action and commonly incites the individual affected to fight or flight This pattern has biologic usefulness since assaults from a hostile environment are likely first to strike the skin Visceral pain on the other hand is characterized by a deeper aching quality which seems to exert a depressing effect, is commonly associated with nausea and followed by inactivity This pattern too appears to have biological significance since fight or flight would be fruitless against assaults from within

It may be possible to distinguish between two different types of pain also with regard to the kinds of stimuli which evoke the sensation Thus the one type is produced mainly by mechanical deformation of the tissues *i e* by pressure stretch distension etc, while the other is more obviously connected with tissue damage which is usually looked upon as the adequate stimulus' for the nociceptive nerve terminals The former type seems to be predominantly represented in deep structures For example distension of a hollow viscus is often associated with pain while the same organ may be cut or burnt with [illegible]
g[illegible]
[illegible]
a[illegible]

Some of the reactions to pain and especially those mediated by the autonom ic nervous system are obviously independent of the perception of the pain since they can be observed in the unconscious individual for example during surgical anesthesia or even in a decerebrate animal These response patterns are consequently not secondary to the psychological phenomenon but can be

mediated *via* neuronal connections at subconscious levels of the central nervous system It is obvious however that higher centres may exert a damping or exaggerating influence upon such reactions The circulatory adjustments initiated by painful stimuli can be included among these autonomic nociceptive reflexes and the differences between cutaneous' and visceral pain as expressed in the quotation above might then be reflected also in their cardiovascular manifestations The pressor effect observed in anesthetized or decerebrate animals when cutaneous afferents especially C fibres are activated would thus represent the reflex circulatory response to noxious stimuli affecting the skin It may perhaps be looked upon as a bulbospinal reflex framework upon which the more differentiated circulatory adjustement of the defence flight reaction as studied by ABRAHAMS HILTON and ZBROZYNA (1960 a b) is superimposed (see Chapter IV B) Deep pain produced by distension compression or distortion of the tissues would be associated with quite an opposite type of cardiovascular response pattern characterized by vagal bradycardia peripheral vasodilatation pooling of blood and fall in blood pressure as exemplified by the observations during abdominal surgery referred to above (FOLKOW *et al* 1962) In view of these considerations it may be said that the concept of group III muscle afferents as being mediators of that kind of deep pain which is produced by pressure tension and blunt trauma seems to be consistent with the circulatory effects that these fibres evoke when artificially stimulated

Summary

Reflex cardiovascular responses to stimulation of somatic afferents were studied in chloralose anesthetized and in decerebrated cats curarized with Flaxedil The circulatory response patterns associated with the reflex blood pressure changes obtained were studied as changes of regional blood flow resistance in skeletal muscle kidney intestine and skin Reflex alterations in heart rate and in regional blood content were also observed Spinal pathways and bulbar structures engaged in the cardiovascular responses to somatic nerve stimulation were studied in experiments with acute lesions in the cervical cord and medulla oblongata

Electrical stimulation of muscle afferents produced reflex blood pressure falls which were particularly marked (40—100 mm Hg) if the prevailing tonic vasoconstrictor fibre activity was high and the buffering capacity of the baroreceptors reduced The depressor effects could be ascribed mainly to activation of group III afferents some observations indicating that group II fibres might contribute to these circulatory responses may be explained by a threshold overlap of fibres functionally more related to those of group III The depressor effects were most pronounced at low stimulation frequencies (5—20 impulses/sec) but were observed also at impulse rates as high as 100—400 impulses/sec In experiments with adequate stimulation blood pressure falls were elicited by squeezing or pinching of the muscle bellies

Stimulation of muscle nerves with intensities strong enough to activate afferent C fibres produced pressor effects at high impulse rates At low frequencies the effect of the depressor afferents was often predominant

Small or insignificant blood pressure falls (10—20 mm Hg) probably due to activation of thin medullated afferents were obtained on electrical stimulation of cutaneous nerves Pressor responses occurred at high stimulus intensities irrespective of the impulse frequency used

In the depressor responses to afferent muscle nerve stimulation a generalized reflex inhibition of tonic sympathetic vasoconstrictor fibre activity produced a vasodilatat on in all the vas

also of capacitance vessels

rate in sympathetic cardio ac [illegible] were other components of this

response pattern Regional quantitative differences in the reflex changes of blood flow resistance could be attributed mainly to differences in the prevailing neurogenic tone of the various vascular beds The influence of the 'somatic depressor reflex' on the general circulatory homeostasis was discussed in the light of its cardiovascular response pattern

The pressor response to high intensity — high frequency stimulation of afferent somatic nerves was accompanied by a pronounced vasoconstriction in the kidney, while this vascular bed was little engaged in the response to carotid artery occlusion In the atropinized animals the muscle vessels showed rather variable degrees of reflex constriction during a 'somatic pressor response' There were some observations indicating that a reflex activation of the cholinergic vasodilator fibres to the skeletal muscles might occur in association with the blood pressure rise The 'somatic pressor reflex' was discussed with regard to its possible relationship to the cardiovascular adjustment of the 'defence alarm reaction'

It was not possible to demonstrate in the present experiments any 'local sign' or segmental organization in the reflex vascular responses studied

As judged from the effect of acute experimental lesions in the cervical spinal cord and medulla oblongata the group III fibres from muscles exert their sympatho inhibitory effect on bulbar cardiovascular centres *via* ascending pathways in the ventral quadrants of the cord The depressor responses to afferent muscle nerve stimulation were abolished by lesions within the ventral part of the bulbar depressor area Ascending pathways mediating the somatic pressor reflex may not be strictly confined to the dorsolateral fasciculi

The physiological significance of the somatic depressor and pressor reflexes was tentatively discussed with regard to the hemodynamic consequences of the cardiovascular adjustments and with regard to the functional role of the responsible afferent fibre systems

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I The effect of body position on the circulation at rest and during exercise with special reference to the influence on the stroke volume
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Acta physiol scand 1962 *56* In press Together with A HOLMGREN and B JONSSON Preliminary report was given at the General Meeting of the Swedish Medical Association in December, 1961

III Postural circulatory changes at rest and during exercise in patients with funnel chest, with special reference to factors affecting the stroke volume
Acta med scand 1962 *171* 695—713 Preliminary report was given at the General Meeting of the Swedish Medical Association in December, 1960

IV Postural circulatory changes at rest and during exercise in five patients with congenital absence of valves in the deep veins of the legs
Acta med scand 1962 *172* 21—29 Together with A LODIN Preliminary report was given at the General Meeting of the Swedish Medical Association in December, 1961

V The effect of cardioacceleration by methylscopolaminenitrate on the circulation at rest and during exercise in supine position with special reference to the stroke volume
Acta physiol scand 1962 *56* In press Preliminary reports were given at the General Meeting of the Swedish Medical Association in December 1959 and 1960

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VII Circulatory response to recumbent exercise and head up tilting in patients with disturbed sympathetic cardiovascular control (postural hypotension) Observations on the effect of norepinephrine infusion and antigravity suit inflation in the head up tilted position
Acta med scand 1962 *172* 623—636 Together with B JONSSON and I KARLOF Preliminary report was given at the General Meeting of the Swedish Medical Association in December 1960

In the text these papers will be referred to by Roman figures I—VII

Preface

This article reviews some investigations performed during the years 1959—1962 at the Laboratory of Clinical Physiology, Thoracic Clinics, and at the Department of Clinical Physiology, Karolinska Sjukhuset, by a group which includes the author Prior to the present investigations the author became interested in circulatory physiology and especially in the circulatory adaptation to exercise and the behavior of the stroke volume to various demands on the circulation The research program at the Department of Clinical Physiology (Head Professor T Sjostrand) has for many years been focused on circulatory and exercise physiology, and the investigations reported in this paper are part of this more extensive program

Investigations of the type reported here can only be performed through team work by a well trained staff I am greatly indebted to the entire staff for the favor of pleasant collaboration For valuable help in various ways I wish to express my sincere thanks to Docent Bengt Jonsson, my chief and Head of the Laboratory of Clinical Physiology, Thoracic Clinics, Professor Torgny Sjostrand, Head of the Department of Clinical Physiology, Docent Alf Holmgren, assistant professor at the Department of Clinical Physiology, Doctor Ingvar Karlof, member of the staff at the Laboratory of Clinical Physiology, Thoracic Clinics, Professor Clarence Crafoord, Head of the Clinic for Thoracic Surgery, Docent Bjorn Nordenstrom Head of the Department of Diagnostic Roentgenology at the Thoracic Clinics, and Professor Henrik Lagerlof, Head of the Medical Clinic Karolinska Sjukhuset

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Stockholm, 1962

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III Postural circulatory changes at rest and during exercise in patients with funnel chest, with special reference to factors affecting the stroke volume
Acta med scand 1962 *171* 695—713 Preliminary report was given at the General Meeting of the Swedish Medical Association in December, 1960

IV Postural circulatory changes at rest and during exercise in five patients with congenital absence of valves in the deep veins of the legs
Acta med scand 1962 *172* 21—29 Together with A LODIN Preliminary report was given at the General Meeting of the Swedish Medical Association in December, 1961

V The effect of cardioacceleration by methylscopolaminenitrate on the circulation at rest and during exercise in supine position, with special reference to the stroke volume
Acta physiol scand 1962 *56* In press Preliminary reports were given at the General Meeting of the Swedish Medical Association in December, 1959 and 1960

VI Observations on the effect of varying ventricular rate on the circulation at rest and during exercise in two patients with an artificial pacemaker
Acta med scand 1962 *172* 615—622

VII Circulatory response to recumbent exercise and head up tilting in patients with disturbed sympathetic cardiovascular control (postural hypotension) Observations on the effect of norepinephrine infusion and antigravity suit inflation in the head up tilted position
Acta med scand 1962 *172* 623—636 Together with B JONSSON and I KARLOF Preliminary report was given at the General Meeting of the Swedish Medical Association in December 1960

In the text these papers will be referred to by Roman figures I—VII

normal subjects with different circulatory dimensions In both older and recent literature discrepancies exist concerning the behavior of the stroke volume during muscular work Some of these discrepancies can be explained by the fact that the influence of body position on the circulatory dynamics was not always recognized Differences in technic and in the materials studied could also account for some of the controversial results With the aim to further elucidate these problems, two series of normal subjects were investigated In one series of nonathletic, healthy males (I) the behavior of the stroke volume was studied at rest and during exercise in the same subject both in the sitting and supine positions In order to study if the circulatory response to exercise was different in subjects adapted to perform heavy exercise during long periods of time, a series of well trained athletes (II) was investigated with identical methods and procedures

In an attempt to collect further information about factors of importance for the ventricular filling and the stroke volume, two groups of patients with apparently normal cardiac function were studied patients with pectus excavatum (III) and patients with congenital absence of venous valves (IV) In contrast to normal individuals, patients with pectus excavatum will at a given heart rate perform a lower work intensity in the sitting than in the supine position In order to determine if this was caused by a lower stroke volume in the sitting position, these patients were studied by the aid of heart catheterization

In previous papers (I, II) it was shown that transition from rest to exercise in the sitting position causes an increase of the stroke volume in normal subjects probably as a result of a redistribution of blood in a central direction For this redistribution the venous pump of the leg muscles is supposed to be important The efficiency of this muscular pump must depend on intact venous valves In an attempt to evaluate the importance of the venous valves for ventricular filling and stroke volume a group of patients with normal cardiac function but with congenital absence of venous valves (IV) were examined at rest and during work in the supine and sitting positions

For our understanding of the general regulation of the blood circulation it is important to study the effect of an artificial variation of heart rate in the intact man during constant oxygen consumption This can be done by pharmacological cardioacceleration with an anticholinergic substance and by artificial variations in ventricular rate in patients treated with pacemakers Animal experiments indicate a reduction of the stroke volume with increasing heart rate Previous studies on man show varying results In the present paper the effect of varying the heart rate has been studied both at rest and during exercise Heart rate has been varied by administration of methylscopolaminenitrate to patients with normal circulation

Introduction

The oxygen transport of the circulation per unit time is equal to the product of heart rate, stroke volume and total arterio-venous oxygen difference as expressed by the Fick equation, which may be written

$$V_{O_2B} + \Delta V_{O_2} = F \cdot SV \cdot AVD \tag{1}$$

where V_{O_2B} = basal oxygen uptake, ΔV_{O_2} = increase in oxygen uptake above basal value, F = heart rate, SV = stroke volume and AVD = total arterio-venous oxygen difference. If an aerobic work involves large muscle groups, as during leg exercise on a bicycle ergometer, and if the rate of work is high enough to increase the total oxygen uptake above one l/min the mechanical efficiency varies only within narrow limits both within and between individuals (WAHLUND 1948, ÅSTRAND 1952). The external rate of work (W) may then be written $W = \Delta V_{O_2} \cdot K_{Me}$, where K_{Me} = = factor which includes the calorific value of oxygen, a thermodynamic equivalent and the mechanical efficiency. By substituting this expression of ΔV_{O_2} into equation (1), the external rate of work performed by a subject may be written

$$W = K_{Me} (F \cdot SV \cdot AVD - V_{O_2B}) \tag{2}$$

This equation shows that the rate of work performed by a subject at a given heart rate is predominantly determined by the stroke volume and the average peripheral oxygen utilization.

Information about the cardiovascular responses to various circulatory demands in normal subjects is a prerequisite for an evaluation and interpretation of changes induced by disease. Our understanding of the circulation must be based on circulatory data obtained in normal subjects. Cardiac catheterization provides the means for more complete information. Its application to normal subjects may be justified under careful precautions, since it has been proven to be a technic which does not involve any undue risks.

The principal aim of the present investigations was to study the variables of the above mentioned equations in normal subjects during various demands on the circulation such as exercise, change of body position and artificial variation in heart rate.

Survey of the literature disclosed a lack of exact knowledge concerning the effectuation of the increasing oxygen transport during exercise in

$PWC_{1\,0}$ was obtained by inter- or extrapolation The *total amount of hemoglobin* (*THb*) was determined by the alveolar CO method (SJOSTRAND 1948), and the *blood volume* was calculated from the *THb* and the hemoglobin concentration of finger blood *Heart volume* was determined in the prone position with exposure during quiet breathing (LARSSON and KJELLBERG 1948) *Cardiac output* was determined according to the direct Fick method with analysis of blood gases spectrophotometrically and of expired air, collected in Douglas bags, according to HALDANE and PRIESTLY (1935) The *breathing valves* used for collection of expired air were of low resistance type For studies of athletes a Hans Rudolph high velocity valve was used With this valve a flow rate of 200 l/min can be achieved with a pressure difference over the valve of 4 cm H_2O A total number of 368 cardiac output determinations were performed with the direct Fick method In paper V and VII cardiac output and central blood volumes were also determined by the *dye dilution technic* Cardiogreen was used as indicator *Blood pressures* were transmitted to a Swema Elema straingauge manometer or an Elema pressure transducer of the variable inductance type, connected to amplifier units Tracings were obtained on an Elema "Klinik" photokymograph (cf HOLMGREN 1956) The linearity and frequency response of the recording system were regularly checked Before and after each pressure tracing, zero and standard pressures were recorded Mean pressures were obtained by means of electric integration. The reference level for zero pressure in the supine position was taken at 5 cm below the sternum at the insertion of the fourth rib, and in cases with funnel chest at 5 cm below the highest level of the fourth rib In paper VII, when the patients were studied in tilted positions, zero pressure was measured at the midthoracic point of an antero-posterior plane through the fourth intercostal space In the sitting position zero pressure was measured at a level through the fourth costal insertion at the sternum

Lactate concentration of the arterial blood was determined according to BARKER and SUMMERSON (1941), as modified by STROM (1949)

Statistical methods Current statistical methods have been used for the calculation of arithmetical mean ($\bar{X}$) standard deviation ($S\,D$), standard error of the mean ($e_{\bar{X}}$), for t analysis and for studies of relationships with linear regression analysis (cf SNEDECOR 1959, KEMP and NIELSEN 1959)

The *general experimental procedure* started with determinations of some measures of the circulatory system such as total amount of hemoglobin, total blood volume, heart volume and rate of work at pulse rate 170 A few days after these determinations heart catheterization was performed All volunteers and patients were informed about the procedures and were familiar with the use of a breathing valve The volunteers received no premedication before heart catheterization Most of the hospital patients were premedicated with a small dose of a sedative, either meprobamate or a barbiturate All investigations were performed without complications

(V) and by varying the stimulating frequency in patients with an artificial pacemaker (VI)

In postural hypotension the *sympathetic cardiovascular control is* impaired. This is thus a condition in which the significance of these regulatory mechanisms during various circulatory demands may be studied Using heart *catheterization, only* few studies of the hemodynamics in postural hypotension have been reported The present investigation (VII) of the effect of exercise and head-up tilting also includes observations on the effect of infusion of the sympathetic transmittor substance, norepinephrine, and of *application of an inflated* antigravity suit in the head up position

Material

The author's findings are based on data from 34 healthy, adult, male volunteers [26 nonathletes (I, V) and 8 well trained athletes (II)], 15 patients with normal circulation (V), 16 patients with funnel chest (III), 5 patients with congenital absence of valves in the deep leg veins (IV), 4 patients *with postural hypotension (VII) and 5 patients with artificial* pacemakers (VI) A total number of 79 individuals was studied, of whom 62 were submitted to cardiac catheterization and 3 were catheterized twice. Detailed information, including tables with anthropometric data, has been *reported in each separate study*

Methods and general procedure

Detailed descriptions of methods and procedures have been reported in the separate papers and are therefore only *briefly summarized below* The different *methods were checked repeatedly* throughout the investigations

ECG and *orthostatic* tests, using CR leads were performed according to the technic described by HOLMGREN *et al* (1957 and 1959) *Spirometric examination* was made according to the method described by HOLMGREN (1954) The *physical working capacity* (*PWC*) was determined in the sitting and recumbent positions according to SJÖSTRAND (1947) and WAHLUND (1948) *on an electrically* braked bicycle ergometer (HOLMGREN and MATTSSON 1954) with a work load increasing stepwise every 6 minutes The PWC_{170} is defined as the rate of work in kpm/min which the *subject can perform* at pulse rate 170 Using the approximately linear relationship between pulse rate and work load, the value of the

PWC_{170} was obtained by inter- or extrapolation The *total amount of hemoglobin (THb)* was determined by the alveolar CO method (SJOSTRAND 1948), and the *blood volume* was calculated from the *THb* and the hemoglobin concentration of finger blood *Heart volume* was determined in the prone position with exposure during quiet breathing (LARSSON and KJELLBERG 1948) *Cardiac output* was determined according to the direct Fick method with analysis of blood gases spectrophotometrically and of expired air, collected in Douglas bags according to HALDANE and PRIESTLY (1935) The *breathing valves* used for collection of expired air were of low resistance type For studies of athletes a Hans Rudolph high velocity valve was used With this valve a flow rate of 200 l/min can be achieved with a pressure difference over the valve of 4 cm H_2O A total number of 368 cardiac output determinations were performed with the direct Fick method In paper V and VII cardiac output and central blood volumes were also determined by the *dye dilution technic* Cardiogreen was used as indicator *Blood pressures* were transmitted to a Swema-Elema straingauge manometer or an Elema pressure transducer of the variable inductance type, connected to amplifier units Tracings were obtained on an Elema "Klinik" photokymograph (cf HOLMGREN 1956) The linearity and frequency response of the recording system were regularly checked Before and after each pressure tracing zero and standard pressures were recorded Mean pressures were obtained by means of electric integration The reference level for zero pressure in the supine position was taken at 5 cm below the sternum at the insertion of the fourth rib, and in cases with funnel chest at 5 cm below the highest level of the fourth rib In paper VII, when the patients were studied in tilted positions, zero pressure was measured at the midthoracic point of an antero posterior plane through the fourth intercostal space In the sitting position zero pressure was measured at a level through the fourth costal insertion at the sternum

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I Effect of exercise in ordinary subjects (I, V)

A Previous investigations

The increase in cardiac output during muscular work was previously considered to depend on continuous increase in both stroke volume and heart rate This concept, based on the application of Starling's law of the heart (Patterson, Piper and Starling 1914) to intact man, is supported by observations with indirect estimations of cardiac output by gasometric technics (Krogh and Lindhard 1912, Lindhard 1915, Christensen 1931 Jorgensen 1954, Brandi and Brambilla 1961) However, a continuous increase in stroke volume during work was no constant finding (Boothby 1915, Douglas and Haldane 1922, Christensen 1931, Nielsen 1937) On the contrary, the majority of investigations during recent years have not shown any marked increase in the stroke volume during work, apparently irrespective of the method used for determination of cardiac output (Riley *et al* 1948, Dexter *et al* 1951, Asmussen and Nielsen 1952, Slonim *et al* 1954, Donald *et al* 1955, Barratt Boyes and Wood 1957 Holmgren, Jonsson and Sjostrand 1960, Wang Marshall and Shepherd 1960, Braunwald and Kelly 1960, Reeves *et al* 1961 a b) In reviews by Sjostrand (1956), Asmussen and Nielsen (1958—59) and by Rushmer and Smith (1959) this has also been noted On the other hand however, some recent studies show an increase in the stroke volume during work (Jorgensen 1954, Musshoff *et al* 1959, Chapman Fisher and Sproule 1960) In some studies a slight to moderate increase in stroke volume was found in some individuals especially on transition from rest to exercise (Warner *et al* 1953, Varnauskas 1955, Muller 1959, McGregor Adam and Sekelj 1961)

B Present investigations

The circulatory adaptation to exercise was studied with heart catheterization in 22 nonathletic subjects with normal circulatory function This material comprises 10 healthy, adult, male volunteers (paper I) and 12 patients (6 females and 6 males in paper V) The majority of these patients were referred to the hospital because of systolic murmurs over the pulmonary area detected at routine examinations The murmurs were evaluated as being of physiological origin and in none of the cases could any heart disease be detected The age varied between 14 and 41 years Most subjects performed two work loads and the pulse rate at the highest load was between 150 and 160 beats min Up to the submaximal work loads studied, cardiac output increased as a rectilinear function of the oxygen consumption This implies that the arterio venous oxygen difference is

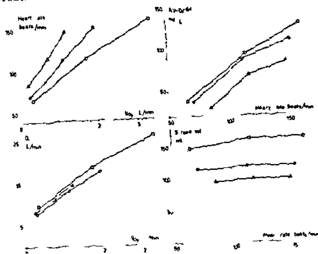


Fig 1 The eff... relations Me... HOLMGREN e... athletes (□) Q = cardiac output, V_{O_2} = oxygen intake

a hyperbolic function of the oxygen uptake In the supine position the cardiac output (Q l/min) was found to increase during work in relation ... V_{O_2} + ...uation, HOLM- ... normal subjects the cardiac output on the average increases by about 6 l/min in order to effectuate an increase in the oxygen transport of 1 l/min As evident from Fig 1, a series of nonathletic, healthy males and a series of nonathletic, healthy females appeared to follow approximately the same regression, which means that the lower oxygen transport capacity of the female group at a given heart rate is mainly due to their lower stroke volume If the cardiac output increases during work as a rectilinear function of the oxygen uptake and with an approximately constant stroke volume, the arterio-venous oxygen difference will increase as a hyperbolic function of the heart rate At a given heart rate during work the arterio-venous oxygen diff... then be high... that the indi... and oxygen ...

In the supine position the increase in cardiac output on transition from rest to exercise and during continued work with increasing load was explained almost exclusively by the rise in heart rate, i e, the stroke

volume was not significantly changed, although it was on the average slightly larger with the first work load than at rest (Fig 1) During work the stroke volume was constant in the sitting position as well

Conclusions

1 The data are in general agreement with the majority of recent investigations on the central circulation (cf HOLMGREN *et al* 1960) and indicate that the increase in cardiac output during muscular work is almost exclusively due to the increase in heart rate In other words, the stroke volume is markedly constant during work of increasing intensity, and of about the same size as at rest in recumbent position
2 The difference in oxygen transport capacity at a given heart rate between a group of healthy males and a group of healthy females was due to the difference in stroke volume

2 Effect of exercise in athletes (II)

A Previous investigations

Some discrepancies in the literature concerning the behavior of the stroke volume during work could be due to differences in material In fact, many of the earlier studies, performed with indirect technics and showing an increase of the stroke volume during work, were carried out on athletic subjects (HENDERSSON, HAGGARD and DOLLEY 1927, CHRISTENSEN 1931, JØRGENSEN 1954) The divergency of their results as related to the results of those using the direct Fick or indicator dilution methods are, therefore, not necessarily explained by inadequate technics Also, recent studies with the direct Fick method have shown a more marked increase of the stroke volume in athletes than in nonathletes MUSSHOFF *et al* (1959) found the stroke volume to increase by 60 % in athletes and by 39 % in ordinary males, and no significant increase in females However, other studies of athletes do not show this marked increase (FREEDMAN *et al* 1955 WANG *et al* 1961) RUSHMER (1959) suggests from data in the literature that athletes probably may show an increasing stroke volume during work

B. Present investigations

In order to study the mechanism of the high oxygen transport capacity in athletes, a series of 8 very well trained cyclists was investigated at rest and during work at 800 and 1600 kpm/min They were characterized by large dimensions of the circulatory system, as reflected in a large total amount of hemoglobin, a large blood volume in relation to body weight and a large heart volume, proportional to the large total amount of hemoglobin The oxygen transport capacity, expressed as the rate of work performed at heart rate 170, was considerably greater than in the nonathletes The cardiac output at rest in recumbent position showed the same relation to the oxygen uptake as in nonathletes, *i e*, the arterio-venous oxygen difference was about equal The arterio-venous oxygen difference at rest, recumbent, averaged 37 ml/l in the nonathletic volunteers (I), 39 ml/l in the athletic volunteers (II) and 32 ml/l in the group of nonathletic patients (V) The low value in the patients with normal circulation can be explained by their greater deviation from basal conditions, as evaluated from the heart rate This might result in a large cardiac output In the athletes the heart rate at rest was 17 % higher than during basal conditions prior to heart [illegible]

[illegible] put and oxygen uptake calculated from data in normal, nonathletic subjects (HOLMGREN *et al* 1960, paper I) At the highest load in recumbent position (1600 kpm min) the oxygen uptake increased to 3364 *ml/min* and the cardiac output reached an average value of 26 3 l/min (Fig 1) The individual values then scattered around the extrapolated regression line found for ordinary subjects The stroke volume at rest, recumbent, averaged 150 ml and increased on transition from rest to exercise by 13 ml, or 9 %, to an average of 163 ml This increase is significant ($p < 0\,001$) There was no further change in *stroke* volume with the heavier load (Fig 1) Also in sitting position the stroke volume was constant once exercise had begun Both at rest and during work the stroke volume was considerably larger in athletes than in nonathletes At a given heart rate the larger oxygen transport capacity of these athletes as compared to nonathletes was explained by a larger stroke volume As evident from Fig 1 the cardiac output during the first work load was even larger for a given oxygen transport value than in nonathletic males Thus, the peripheral oxygen utilization was not higher in relation to the oxygen uptake During exercise the pulmonary arterial wedge pressure and right ventricular end diastolic pressure were higher than in nonathletes There was no decrease in right ventricular end-diastolic pressure with exercise as observed in nonathletes The mean pressure in the brachial artery in relation to cardiac index followed approximately the same regression line

as in nonathletes; i.e., the calculated total peripheral vascular resistance decreased to about the same extent with increasing work load in both groups. The mean pressure in the pulmonary *artery was higher in the* athletes and in proportion to their higher pulmonary arterial wedge pressure. The calculated resistance in the pulmonary vascular bed decreased to about the same extent with exercise as in nonathletes.

Conclusions

1. The increase in cardiac output for a given increase in oxygen transport during work was approximately the same as in nonathletes, although the athletes had a somewhat larger cardiac output for a given oxygen transport value during the first work load. In other words, the *peripheral oxygen utilization was not higher in relation* to oxygen uptake.
2. Therefore, the larger *oxygen transport capacity* of these athletes as compared to nonathletes was only explained by a larger stroke volume.
3. In recumbent position the stroke volume showed a slight but significant increase (9 %) on transition from rest to work. After initial changes the stroke volume remained constant during continued work with a heavier load both *in the supine and sitting positions.*
4. During work the end-diastolic pressure of the right ventricle and the pulmonary capillary venous *pressure were higher than in* healthy nonathletes.

3. Effect of body position at rest and during work in normal subjects (I, II)

A. Previous investigations

The contradictory results reported *in the earlier literature* concerning the behavior of the stroke volume during exercise could to *some* extent be explained *by the fact that some studies were made in* the supine and some in the upright posture. The effect of body position was generally not taken into account. In several of the earlier reports the subjects were studied in a sitting or semirecumbent position. This might partly explain the results obtained by CHRISTENSEN (1931) and JORGENSEN (1954). MITCHELL, SPROULE and CHAPMAN (1958) *studied subjects in the erect position and* then found a two-fold increase of the stroke volume from rest to exercise.

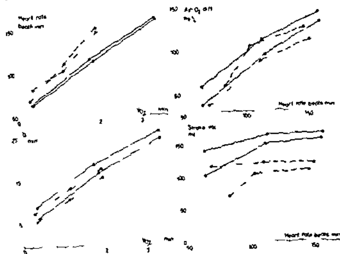


Fig 2 The effect of body position at rest and during work with two progressive load on some hemodynamic relations Mean values for 8 well trained athletes (full lines) and 8 male nonathletes (broken lines) ● = supine position O = sitting position Other symbols as in Fig 1 The mean values at rest for the athletes comprise only 5 subjects, since in 3 cases no values were obtained at rest in the sitting position

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B Present investigations

The effect of body position on the circulation at rest and during work at two successive loads was studied with the aid of cardiac catheterization in a group of 8 nonathletic healthy *male volunteers* and in a group of 8 male athletes Both in athletes and nonathletes the cardiac output was about 2 l/min less in the sitting than in the supine position at rest, as well as during moderate and heavy *exercise (Fig 2)* This difference was due to a smaller stroke volume *in the sitting* position The decrease in stroke volume on transition from supine to sitting position amounted to 46 ml in the nonathletes and to 43 *ml in the* athletes. It was thus of the same absolute order in both groups, but relatively less important in the athletes in relation to their larger stroke volume and blood volume In the sitting position the stroke *volume then* increased considerably with mild work, but showed no further significant increase with heavier loads The stroke volume in the *sitting position* never reached the values obtained

as in nonathletes, *i e*, the calculated total peripheral vascular resistance decreased to about the same extent with increasing work load in both groups The mean pressure in the pulmonary artery *was higher in the* athletes and in proportion to their higher pulmonary arterial wedge pressure The calculated resistance in the pulmonary vascular bed decreased *to about the same extent with exercise as in nonathletes*

Conclusions

1 The increase in cardiac output for a given increase in oxygen transport during work was approximately the same as in nonathletes, although the athletes had a somewhat larger cardiac output for a given oxygen transport value during the first work load In other words, the peripheral oxygen utilization was not higher in relation to oxygen uptake
2 Therefore, *the larger oxygen transport capacity of these athletes* as compared to nonathletes was only explained by a larger stroke volume
3 In recumbent position the stroke volume showed a slight but significant *increase (9 %)* on *transition from rest to work* After initial changes the stroke volume remained constant during continued work with a heavier load both in the supine and sitting positions
4 During work the end diastolic pressure of the right ventricle and the pulmonary capillary venous pressure were higher than in healthy non athletes

3 Effect of body position at rest and during work in normal *subjects (I, II)*

A. Previous investigations

The contradictory results reported in the earlier literature concerning the behavior of the stroke volume during exercise could to some extent be explained by the fact that some studies were made in the supine and some *in the upright posture* The effect of body position was generally not taken into account *In several of the earlier reports the subjects were studied in* a sitting or semirecumbent position This might partly explain the results *obtained by* CHRISTENSEN (1931) and JØRGENSEN (1954) MITCHELL, SPROULE and CHAPMAN (1958) studied subjects in the erect position and then found a two-fold increase of the stroke volume from rest to exercise

3 A higher arterio venous oxygen difference in the sitting position compensates for the lower stroke volume in this posture, so that during work the oxygen transport per heart beat is equal which explains why the capacity to perform muscular work at a given heart rate is independent of posture in normal subjects

4 Relationships between stroke volume, heart volume, total amount of hemoglobin and capacity for work in normal subjects

A Previous investigations

Correlations between size and function of the cardiovascular system have been studied by several authors (KJELLBERG, RUDHE and SJOSTRAND 1949, ÅSTRAND 1952, SJOSTRAND 1953 a, b 1955, REINDELL *et al* 1957, HOLMGREN *et al* 1960 ROSKAMM *et al* 1961) SJOSTRAND (1953 a) stressed that the heart volume should be related to the functional capacity and blood volume and not to body surface area This concept is important, not only because it includes a biological meaning but also because it more clearly defines the borderlines between normal and abnormal HOLMGREN *et al* (1960) demonstrated that the stroke volume was directly related in a linear fashion to the heart volume total amount of hemoglobin and the rate of work developed at heart rate 170 on a bicycle ergometer

B Present investigations

The results reported in this paper have further corroborated the close relationships between size and function of the cardiovascular system For statistical analysis of these relationships in normal nonathletic subjects the data of 14 males and 4 females published by HOLMGREN *et al* (1960) and the data of 10 males reported in paper I were used The physical working capacity at heart rate 170 (PWC_{170} kpm/min), determined on a bicycle ergometer in recumbent position during heart catheterization, could be expressed as a linear function of the stroke volume (SV ml) according to the equation $PWC_{170} = 10\ 8\ \ SV - 311$ ($S\ D = 116$ $r = 0\ 88$, $n = 27$) As suggested by HOLMGREN *et al* (1960) the mean value of the stroke volume during work was used in order to diminish the random variations The athletes had on the average a higher capacity

in the supine position, even during heavy work, when the difference was on the average 11 ml in nonathletes and 9 ml in athletes From the viewpoint of oxygen transport, this difference in stroke volume between the two body positions is compensated for by a higher arterio-venous oxygen difference in the sitting position, so that the oxygen transport per pulse beat is equal (Fig 2) In other words, the rate of work performed at heart rate 170 was independent of the body position both in athletes and nonathletes

In the athletes there was a less marked decrease of right ventricular filling pressure after changing posture from supine to sitting, corresponding to the relatively smaller change in stroke volume With the assumption that the difference between the end-diastolic and the initial-diastolic pressures in the right ventricle is an expression of the effective filling pressure, the values so obtained in the athletes may indicate a lower effective filling pressure at rest in the sitting position than in the supine position The values also indicate an increase of the effective filling pressure with exercise, more so in the sitting than in the supine position

The influence of body position on the central circulation may be explained by variations in blood distribution A change of posture from recumbency to erect position causes a shift of blood from the thorax to the lower part of the body (SJOSTRAND 1941, LAGERLOF *et al* 1951, SJOSTRAND 1953 a) This results in a decrease of heart volume (JONSELL and SJOSTRAND 1941, NYLIN 1934, HOLMGREN and OVENFORS 1960) and stroke volume, probably as a result of lower effective filling pressure On transition from rest to leg exercise in the erect position both heart volume (HOLMGREN and OVENFORS 1960) and stroke volume increase to levels slightly below those obtained in supine position This seems to indicate a redistribution of blood in a central direction The influence of body position during work in normal individuals has been the subject of some recent reports (REEVES *et al* 1961 a, b, MCGREGOR, ADAM and SEKELJ 1961) These independent studies from different laboratories are in general agreement

Conclusions

1 The cardiac output is less in the sitting than in the supine position by about 2 l/min in healthy adult males

2 The stroke volume at rest is substantially smaller in the sitting than in the supine position It increases considerably with mild leg exercise in the sitting position and then remains essentially constant during continued exercise with a heavier load, but on a slightly lower level than in the supine position

3. A higher arterio-venous oxygen difference in the sitting position compensates for the lower stroke volume in this posture, so that during work the oxygen transport per heart beat is equal, which explains why the capacity to perform muscular work at a given heart rate is independent of posture in normal subjects.

4. Relationships between stroke volume, heart volume, total amount of hemoglobin and capacity for work in normal subjects

A. Previous investigations

Correlations between size and function of the cardiovascular system have been studied by several authors (KJELLBERG, RUDHE and SJÖSTRAND 1949, ÅSTRAND 1952, SJÖSTRAND 1953 a, b, 1955, REINDELL *et al.* 1957, HOLMGREN *et al.* 1960, ROSKAMM *et al.* 1961). SJÖSTRAND (1953 a) stressed that the heart volume should be related to the functional capacity and blood volume, and not to body surface area. This concept is important, not only because it includes a biological meaning but also because it more clearly defines the borderlines between normal and abnormal. HOLMGREN *et al.* (1960) demonstrated that the stroke volume was directly related in a linear fashion to the heart volume, total amount of hemoglobin and the rate of work developed at heart rate 170 on a bicycle ergometer.

B. Present investigations

The results reported in this paper have further corroborated the close relationships between size and function of the cardiovascular system. For statistical analysis of these relationships in normal, nonathletic subjects the data of 14 males and 4 females published by HOLMGREN *et al.* (1960) and the data of 10 males reported in paper I were used. The physical working capacity at heart rate 170 (PWC_{170} kpm/min.), determined on a bicycle ergometer in recumbent position during heart catheterization, could be expressed as a linear function of the stroke volume (*SV* ml) according to the equation $PWC_{170} = 10.8 \cdot SV - 311$ (S.D. = 116, $r = 0.88$, $n = 27$). As suggested by HOLMGREN *et al.* (1960) the mean value of the stroke volume during work was used in order to diminish the random variations. The athletes had on the average a higher capacity

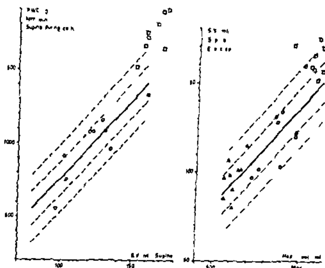


Fig 3 *Left panel* physical working capacity at heart rate 170 (PWC_{170}), determined during heart catheterization in the supine position, in relation to the mean value of the stroke volume during work (S V) *Right panel* stroke volume, mean value during supine exercise, in relation to heart volume at rest in the prone position. Regression lines ± one and two standard errors of estimate obtained from data of 27 healthy subjects, are denoted (materials and equations see text) Values for females (△), healthy, nonathletic males (O) and athletes (□) Data of 4 females reported by HOLMGREN *et al* (1960) are also plotted

for work at heart rate 170 than predicted from their stroke volume according to the regression line for ordinary subjects (Fig 3). This deviation *is at least partly to be expected, because they can with their larger stroke* volume reach a greater cardiac output and higher arterio-venous oxygen difference at a given heart rate A close correlation was also obtained between the capacity for work, determined at heart rate 170 (PWC_{170} kpm/min) in the sitting position before heart catheterization, and the mean value for the stroke volume (SV ml) during recumbent exercise $PWC_{170} = 10.0 \quad SV - 195$ ($S\ D = 129$, $r = 0.84$, $n = 27$) The close similarity between these two regression equations is explained by the fact that the PWC_{170} was on the average independent of body position and not significantly influenced by the cardiac catheterization The variation in PWC_{170} *for a given value of the stroke volume is, except for methodological errors,* mainly explained by variations in the arterio-venous oxygen difference, since the *variations in mechanical efficiency between individuals are relatively small in normal subjects The stroke volume* (SV ml) during recumbent exercise was also linearly correlated to the heart volume at rest in the prone position (HV ml) according to the equation $SV = 0.111 \cdot HV + 25.9$ ($S\ D = 11.1$, $r = 0.83$, $n = 27$) and to the total amount of hemoglobin (THb g) according to the equation: $SV = 0.103 \cdot THb + 43.9$ ($S\ D = 9.8$; $r = 0.79$, $n = 27$). In relation to the heart

volume the stroke volume in the athletes was larger than predicted from the regression line found for ordinary subjects (Fig 3) This deviation might be explained by the fact that these athletes were a selection of the best among a group of athletes and thereby possessed a more efficient adaptation to muscular work than other subjects with the same circulatory dimensions

Conclusions

1 The present investigations on normal subjects have further demonstrated that close correlations exist between stroke volume on one hand and heart volume total amount of hemoglobin and capacity for work at a given heart rate on the other

2 A selected group of athletes may show a more efficient adaptation to muscular work than expected from their circulatory dimensions

5 Effect of body position and exercise in patients with funnel chest and orthostatic pulse reaction during work (III)

A Previous investigations

Funnel chest (pectus excavatum) is a congenital thoracic deformity in which the lower part of the sternal body and the adjacent parts of the ribs are displaced towards the spine thus diminishing the space in the lower part of the thorax Some investigators (WACHTEL, RAVITCH and GRISHMAN 1956, LESTER 1958) are of the opinion that this deformity may have serious consequences on cardiac function This opinion is not supported by other studies using cardiac catheterization (FABRICIUS DAVIDSEN and HANSEN 1957, BERGH and BERGLUND 1961) A reduced exercise tolerance is reported to occur with this deformity (LESTER 1950, HOWARD 1959) This opinion is not based upon objective measurement, however, and it has not been analysed whether a decreased physical working capacity is caused by cardiac or respiratory factors

It was observed in our laboratory some years ago that in cases with funnel chest the rate of work performed at pulse rate 170 was often substantially higher in the supine than in the sitting position, whereas there is generally no such difference in normal subjects Since the effect

of body position on the circulation at rest and during leg exercise had been analysed in normal subjects (paper I) it was consequently of interest to make a similar study in the patients with funnel chest showing an orthostatic pulse reaction during leg exercise

B Present investigations

Sixteen patients with funnel chest were studied and the results were compared with data from a series of normal subjects similarly investigated (paper I) The thoracic deformity was pronounced in all cases The distance from the posterior surface of the body of the sternum to the anterior border of the vertebral body as measured from the X ray, varied between 3 5 and 9 5 cm (mean value = 6 0 cm) The rate of work performed on a bicycle ergometer at pulse rate 170 was significantly lower in the sitting than in the supine position The difference averaged 15 % of the supine value The respiratory function appeared to be unimpeded during submaximal work as judged by clinical observation and the normal arterial oxygen saturation values Eleven cases were studied with the aid of right heart catheterization at rest and during exercise both in the sitting and supine positions No evidence of heart disease was found in any of the cases The central circulation was normal in the supine position Thus there were no signs that the depressed sternum caused any restriction of diastolic expansion of the ventricles since a normal stroke volume was maintained with normal ventricular filling pressure in the recumbent position As in normal subjects the stroke volume at rest was 40 % smaller in the sitting than in the supine position On transition from rest to work in the sitting position the stroke volume increased significantly less than in normal subjects (Fig 4) The stroke volume during work was thus 31 % smaller in the sitting than in the supine position compared to an average difference of 12 % for nonathletic normal subjects Due to a correspondingly more marked increase in heart rate however the cardiac output rose normally in relation to the oxygen uptake during work in the sitting position The lower rate of work at a given heart rate in the sitting than in the supine position was thus explained by the smaller stroke volume (Fig 4) The insufficient increase in stroke volume on transition from rest to leg exercise in the sitting position is probably explained by impaired ventricular filling It is reasonable to assume that this circulatory disturbance is caused by the obvious thoracic deformity which encroaches upon the volume of the lower part of the thorax Preliminary studies on two cases have shown that this abnormal postural effect on the stroke volume during work is not abolished by inflation of an anti gravity suit worn by the patient This observation lends further support to the view that there is a direct causal connection between the thoracic

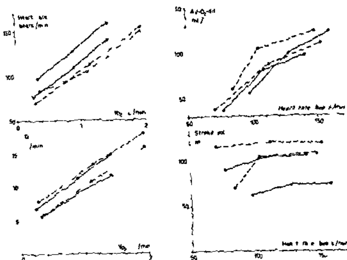


Fig 4 The effect of body position at rest and during work at two progressive loads on some hemodynamic relations Mean values for 11 patients with funnel chest (full lines) showing an orthostatic pulse reaction during work, and for 8 healthy, nonathletic males (broken lines) ● = supine position, ○ = sitting position Other symbols as in Fig 1

deformity and the circulatory disturbance Angiocardiography performed in four cases has not revealed any signs of anatomical impediment of blood flow in the caval veins that could cause an abnormal distribution of the blood vol

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small and revealed a visible impression from the thoracic deformity. With increasing heart rate, as during exercise, diastole shortens, and atrial systole will contribute to ventricular filling in a progressively larger amount A small volume of the right atrium could influence the filling of the right ventricle proportional to the importance of atrial systole for ventricular filling, and after some beats also the filling of the left ventricle Such a mechanism could be significant if the heart in the upright posture takes such a position that the right atrium comes to rest upon the depressed sternum and therefore is altered in shape

It is also possible that the deformity could interfere with the efficiency of the abdominothoracic pumping mechanism, which is important for venous inflow to the thorax as demonstrated in animals (BRECHER 1956) This mechanism might be more important du

diaphragm has not yielded any significant information concerning the possibility of such a mechanism. Further studies are necessary to ascertain the underlying mechanism of this abnormal reaction to posture.

Conclusions

1. An orthostatic pulse reaction during leg exercise occurs in patients with pectus excavatum and may reduce considerably the capacity for work at a given pulse rate in the sitting position.
2. This is explained by a deficient ability to increase the stroke volume on transition from rest to leg exercise in the erect position.
3. An impaired ventricular filling during work in the erect position can explain this abnormal postural influence on the stroke volume.
4. This investigation has not elucidated the underlying mechanism of the circulatory disturbance, but may be suggestive of an impression on the right atrium by the depressed sternum in the erect position and/or an interference with venous inflow due to impaired efficiency of the abdominothoracic pump.

6. Significance of the valves in the deep leg veins at rest and during work in recumbent and sitting position (IV)

A. Previous investigations

Transition from rest to exercise in the sitting position causes an increase of the heart volume (HOLMGREN and OVENFORS 1960) and the stroke volume (I, II) to levels slightly below those obtained in the recumbent position. This indicates a redistribution of blood to the thorax. The venous pump of the leg muscles is regarded as an important factor for this redistribution. The efficiency of this "muscular pump" must depend on intact venous valves. It has been demonstrated that at rest in the erect position the venous pressure of the lower leg equals that of the hydrostatic pressure up to the level of the heart, and that intermittent contraction of the leg muscles reduces the mean venous pressure considerably (POLLACK and WOOD 1949, HÖJENSGÅRD and STURUP 1952 a, b). This indicates that intermittent muscular contraction reduces the volume of blood contained in the leg veins and accelerates the venous flow.

B Present investigations

Five patients with congenital absence of venous valves were investigated The circulatory adaptation to leg exercise in the recumbent and sitting positions was studied with the aid of right heart catheterization. These patients were eminently suited for an attempt to analyse the significance of the valves in the leg veins for the circulatory adaptation to different demands, since the general clinical picture was not obscured by varicosities or any previous local disease processes of the veins The clinical findings [illegible] the results [illegible] he phlebo- [illegible] valves in the upper and lower extremities Two cases had a few valves per venous trunk in the lower leg Apart from this anomaly, the patients were all healthy The three patients with complete absence of venous valves showed during leg exercise on the bicycle ergometer a markedly higher pulse rate in the sitting than in the supine position No such difference was noted in the two patients who had a few valves in the leg veins During cardiac catheterization one patient was very anxious and probably as a consequence thereof had a large cardiac output, most marked in the beginning of the investigation With regard to the influence of body position during work, however, the general performance of this patient did not differ from that of the others Except for this deviation, all cases showed an essentially normal central circulation in the recumbent position In the cases with complete absence of venous valves the stroke volume during work was on the average 35 % smaller in the sitting than in the recumbent position This difference is significantly larger than in normal, nonathletic subjects (I) where the average difference was 12 % The considerably smaller stroke volume during work in the sitting position was compensated for by a higher heart rate, so that the cardiac output in this position increased fairly normally in relation to the oxygen uptake On the other hand, with regard to oxygen transport a small stroke volume could be compensated for, within some limits, by an increase in the peripheral oxygen utilization, so that no difference in heart rate occurred, as also illustrated in this small series The small stroke volumes during leg exercise in the sitting position can be explained by an impaired ability to redistribute blood from the legs to the central circulation on transition from rest to exercise in the absence of valves in the leg veins

Conclusions

1 Absence of valves in the leg veins did not appear to influence the central circulation either at rest or during work when the subject was recumbent with the legs slightly elevated above the level of the heart

2 On *transition from rest to exercise* in the sitting position the stroke volume failed to increase in the normal way This can be explained by *a reduced ventricular filling* because of an impaired ability, in the absence of valves in the leg veins, to redistribute gravitationally shifted blood *with onset of leg exercise*

7. Effect of artificially induced variations in heart rate at rest and during work (V, VI)

A Previous investigations

Animal experiments have shown that cardioacceleration with an artificial pacemaker reduces the stroke output (BERGLUND *et al* 1958, RUSHMER 1959, WARNER and TORONTO 1960, MILLER *et al* 1962), apparently independent of whether by atrial or ventricular stimulation ABRAMS, HUDSON and LIGHTWOOD (1960) studied the circulation in one patient during artificial pacemaking at rest and found a decrease of the stroke volume with increasing heart rate Similar results were reported by MULLER and BELLET (1961) in their study of one patient

When the heart rate in man has been increased by administration of an anticholinergic substance, usually atropine, the stroke volume has been found to decrease (BERRY *et al* 1959, GORTEN *et al* 1961) or remain unchanged (WEISSLER, LEONARD and WARREN 1957) In a patient who developed paroxysmal tachycardia during catheterization the author has observed a concomitant decrease of the stroke volume Similar observations have also been made by others (SAUNDERS and ORD 1962)

B Present investigations

The present study was designed to evaluate in man the effect of artificially induced variations in heart rate on the central circulation at rest and during recumbent exercise Two methods were used to change the heart rate while the oxygen consumption was constant (1) cardioacceleration by administration of an anticholinergic substance, (2) variation of the stimulating frequency in patients treated with artificial pacemakers For studies with the first mentioned method which is the only one applicable to intact man methylscopolaminenitrate (MSN) was chosen as the experimental drug because of its marked anticholinergic *properties* In nine healthy, adult males the external rate of work, performed on a bicycle

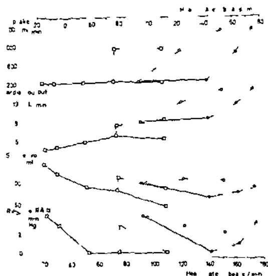


Fig 5 The effect of artificial variations of heart rate at rest (full lines) and during recumbent exercise (broken lines) on oxygen uptake cardiac output, stroke volume and on right ventricular end diastolic pressure (RV_{De}) and right atrial mean pressure (RA) respectively □ = a case with artificial cardiac pacemaker showing typical response to changes in ventricular rate O = mean values for 6 patients with normal cardiac function at rest and during work at two progressive loads ● = mean values at rest and during work for the same group after injection of methylscopolaminenitrate Dotted lines connect values at the same work load before and after cardioaccelerator influence of methylscopolaminenitrate

ergometer at heart rate 170, was determined before and 15—20 min after i m injection of 0 75 mg MSN when the heart rate was stable The heart rate at rest, recumbent, increased 77 % The increase in heart rate on standing was about the same before and after MSN During leg exercise in the sitting position the heart rate was also higher after MSN, but the difference decreased gradually with increasing work load, so that the rate of work performed at heart rate 170 was only 14 % lower after MSN This difference was significant, however The lines connecting heart rate in relation to work load, obtained before and after MSN, tended to converge at the expected level of the maximal heart rate for the age group (ROBINSON 1938, ÅSTRAND 1952) This is in agreement with the results obtained by ROBINSON *et al* (1953), who found no significant effect on the maximal working pulse by atro[illegible]
analysis with cardiac catheterizatio[illegible]
revealed that the increase in heart [illegible]
responding decrease in stroke volu[illegible] at on the average the cardiac output was held constant (Fig 5) The ventricular filling pressures decreased As a consequence of the smaller stroke volume there was a de-

crease of the pressure pulse amplitude in the brachial artery, but the mean pressure was unchanged. The heart volume also tended to decrease after MSN, and on the average with a value approximately corresponding to twice the decrease in stroke volume. These findings are consonant with diminished ventricular filling because of a change in the distribution of the blood volume. However, this view is irreconcilable with the present finding of an unchanged central blood volume, as calculated from dye dilution curves performed by central injection and sampling. The primary interpretation of these data would then reasonably indicate a general diminution of venous tone, provided that the current technic for estimation of the central blood volume from dye dilution curves was valid during the experimental conditions. If the explanation was peripheral pooling of blood, although not detectable with the present method for determination of the central blood volume, the pooled blood does not appear to be available in the large veins, since the studies of BERRY *et al.* (1959) have shown that certain maneuvers intended to redistribute blood, such as head-down tilting and passive leg raising, does not increase the cardiac output or stroke volume after atropine, whereas rapid infusion of serum albumin has a marked effect.

During work performed after MSN the heart rate was higher than during work with the same load before MSN. This difference decreased with increasing load. After MSN the ventricular filling pressures and the stroke volume increased progressively with the work load. The increase of the stroke volume was such that the cardiac output was the same as before MSN at equal oxygen uptake levels (Fig. 5). With the highest work load the stroke volume was only 12 % lower than before MSN at the same load. This is well in accordance with the observed 14 % decrease in the rate of work at heart rate 170. The calculated vascular resistances in the systemic and pulmonary circulations were also unchanged after MSN during work.

After MSN the duration of the mechanical diastole was shorter than at the same heart rates before MSN. It is possible that this contributed to restriction of ventricular filling.

The effect of artificial changes in heart rate was also studied by varying the stimulating frequency in patients treated with artificial pacemakers. The stroke volume was largest at the lowest ventricular rate and decreased with increasing rate, both at rest and during leg exercise at constant oxygen uptake (Fig. 5). The cardiac output at rest was within normal limits in relation to oxygen uptake and relatively constant at ventricular rates within the range of 70 to 110 beats/min. At the lower heart rates studied, the cardiac output decreased to abnormally low values, despite the increasing stroke volume. The cardiac output was thus rate-dependent within the range of 20 to 70 beats/min. The stroke volume was larger during work than at rest at the same ventricular rate. The mean pres-

sure in the right atrium and pulmonary artery changed in the same direction as the stroke volume, both at rest and during work A plausible interpretation of these findings would be that in these cases a change in ventricular rate *per se* can influence the distribution of the blood volume or venous tone and thus also ventricular filling and stroke volume Atrial rate varied inversely with ventricular rate This is reasonably an expression of concomitant changes in sympathetic tone At rest the mean pressure in the brachial artery tended to vary directly with ventricular rate at the lower rates The lack of co ordination between atrial and ventricular contractions cannot be the only explanation for the considerable changes in stroke volume occurring at low heart rates and cannot explain the variations in right atrial mean pressure The underlying mechanism for the demonstrated rate-dependence of the stroke volume in these cases is therefore likely to be explained by a regulatory response elicited *via* the baroreceptors and effectuated *via* the sympathetic outflow

The results obtained by the two methods used for investigation of the circulatory adaptation to an isolated variation in heart rate are in general agreement and indicate that regulatory mechanisms exist by which the size of the stroke volume can be adjusted so that cardiac output is kept fairly constant within the limits previously described This seems also to be true during exercise with unchanged oxygen uptake This is in accordance with the conclusions made by WARNER and TORONTO (1960) from their results of similar experiments on dogs

The present results thus indicate that during artificial variation of the heart rate during constant oxygen consumption the circulation was regulated to maintain a constant mean aortic pressure Since the calculated peripheral vascular resistance was unchanged this means that the regulation acts through the size of the stroke volume

Conclusions

1 During unchanged oxygen uptake an artificial increase in heart rate, either by administration of methylscopolaminenitrate or by augmentation of the stimulating frequency in patients subjected to artificial pacemaking caused a decrease of ventricular filling pressures and stroke volume Within limits the adjustment was such that cardiac output was held fairly constant

2 During recumbent leg exercise, performed after injection of methyl scopolaminenitrate, ventricular filling pressures and stroke volume increased progressively with the load

3 The results indicate that on changing the heart rate selectively, regulatory mechanisms may adjust the size of the stroke volume so that the mean aortic pressure and the total peripheral vascular resistance can be kept fairly constant within limits

crease of the pressure pulse amplitude in the brachial artery, but the mean pressure was unchanged. The heart volume also tended to decrease after MSN, and on the average with a value approximately corresponding to twice the decrease in stroke volume. These findings are consonant with diminished ventricular filling because of a change in the distribution of the blood volume. However, this view is irreconcilable with the present finding of an unchanged central blood volume, as calculated from dye dilution curves performed by central injection and sampling. The primary interpretation of these data would then reasonably indicate a general diminution of venous tone, provided that the current technic for estimation of the central blood volume from dye dilution curves was valid during the experimental conditions. If the explanation was peripheral pooling of blood, although not detectable with the present method for determination of the central blood volume, the pooled blood does not appear to be available in the large veins, since the studies of BERRY *et al.* (1959) have shown that certain maneuvers intended to redistribute blood, such as head down tilting and passive leg raising, does not increase the cardiac output or stroke volume after atropine, whereas rapid infusion of serum albumin has a marked effect.

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On changing from recumbency to a head up tilted position the systemic blood pressure fell markedly, but the heart rate was little changed The cardiac output and the stroke volume decreased abnormally when com pared to a group of healthy old men of comparable body size (GRANATH *et al* 1961) This can be explained by impaired sympathetic adjustment, both of the resistance and of the capacity vessels The exertional hypo tension which is obviously more marked in the erect position seems to be the factor which limits performance of muscular work

By infusion of a very small dose of the sympathetic transmittor sub stance, norepinephrine, when the patient was tilted it was possible to normalize the circulatory response to head up tilting These patients were abnormally sensitive to infusion of norepinephrine, and a marked effect was obtained with a third to a fourth of the minimum amount of nore pinephrine per kg body weight required to produce an increase of blood pressure in normal subjects (GOLDENBERG *et al* 1948) This increased sensi tivity to norepinephrine and also the observation that atropine does not increase the heart rate (BRADBURY and EGGLESTON 1925, CROST and FRIED LANDER 1952) is compatible with a low sympathetic tone in patients with postural hypotension The end diastolic pressure of the right ventricle did not show any definite relation to the increase in stroke volume observed during infusion of norepinephrine With infusion of norepinephrine in a dose producing a very slight increase of blood pressure in normal subjects, these patients revealed a very marked rise In normal subjects there is usually a reflex decrease in heart rate when systemic arterial pressure rises after norepinephrine (GOLDENBERG *et al* 1948) In these patients with postural hypotension, however, this response was reversed, which also indicates that the normal reflex pressor response is impeded With a larger dose of norepinephrine (0 043—0 071 $\mu g/kg/min$) the stroke volume in the head up tilted position reached the same value as in the recumbent position and the cardiac output then exceeded the recumbent value Actions on both resistance and capacity vessels and possibly also on myo cardial contractile force may explain these effects of norepinephrine If part of the mechanism in postural hypotension is due to an abnormally large gravitational displacement of blood, application of pressure to the lower half of the body should relieve the postural circulatory disturbance Application of pressure by means of an antigravity suit proved this to be true in these cases Blood pressure, stroke volume and cardiac output were partially restored to the control value in recumbency The effect was similar to that of a small dose of norepinephrine

Conclusions

1 As judged from studies on patients with postural hypotension, impair ment of the sympathetic cardiovascular control mechanisms did *not*

8 Significance of the sympathetic cardiovascular control as studied on patients with postural hypotension (VII)

A Previous investigations

Postural hypotension, first described by BRADBURY and EGGLESTON (1925) is a rare disturbance of the reflex cardiovascular regulation of arterial blood pressure The underlying lesion is believed to be organic and to have a central localization (ELLIS and HAYNES 1936, STEAD and EBERT 1941 HAMMARSTROM and LINDGREN 1942, NYLIN and LEVANDER 1948, VEREL 1951) Only in a few instances have the hemodynamics of such cases been analysed with the aid of cardiac catheterization (HICKAM and PRYOR 1951 SIEKER *et al* 1956 and SOLOMON and KUHN 1960) or with dye dilution technic (BICKELMAN LIPPSCHUTZ and BRUNJES 1961)

B Present investigations

Four patients with postural hypotension were investigated with cardiac catheterization The purpose was to study the effect of the impaired sympathetic cardiovascular control in these cases on the central circulation during different demands, such as recumbent exercise and tilting Observations on the effect of norepinephrine infusion and antigravity suit inflation in the tilted position were included In the supine position the cardiac output was normal in relation to the oxygen uptake at rest and increased normally during work when compared to a group of healthy old men (GRANATH, JONSSON and STRANDELL 1961) There were no signs of impaired ventricular filling in the recumbent position either at rest or during exercise with the legs slightly elevated above the level of the heart The systemic blood pressure decreased abnormally during recumbent exercise This indicates an impaired reflex control of the resistance vessels Analogous results were obtained in animal experiments performed by VON EULER and LILJESTRAND (1946) who found a decrease in systemic blood pressure during electrically induced work when the baroreceptors had been denervated MARSHALL SHIRGER and SHEPHERD (1961) also reported in patients with postural hypotension an exertional hypotension during work in the reclining position The vasodilatation elicited in the active muscles during work is apparently not compensated for by reflex adjustment of the total peripheral vascular resistance Therefore the calculated peripheral resistance showed an abnormally steep decrease with exercise

during continued exercise with a heavier load as in the supine position, but on a slightly lower level This is true both for athletic and nonathletic young subjects

5 With regard to oxygen transport, the smaller stroke volume in the sitting position is compensated for by a higher peripheral oxygen utilization, so that during work the oxygen transport per heart beat is equal in both postures In other words, the capacity for work at a given heart rate is independent of body position in normal subjects

6 The capacity for work at a given heart rate is dependent on body position in patients with pectus excavatum and is significantly lower in the sitting position This is explained by a deficient increase of the stroke volume on transition from rest to work in the sitting position The stroke volume is thus abnormally small during work in the sitting position The underlying mechanism could be that in the sitting posture the right atrium rests on the depressed sternum, which then exerts a compressive effect, and/or an impaired efficiency of the abdominothoracic pumping mechanism

7 In the absence of valves in the leg veins, the stroke volume may fail to increase to normal extent on transition from rest to leg exercise in the erect position because of impaired ability to redistribute the gravitationally shifted blood This may reduce the capacity for work in the erect position

8 During constant oxygen uptake an artificially induced increase in heart rate, either by injection of an anticholinergic substance (methylscopolaminenitrate) or by raising the stimulating frequency to the ventricles in patients treated with an artificial pacemaker, caused a decrease of ventricular filling pressures and stroke volume If cardioacceleration by methylscopolaminenitrate was followed by exercise, the ventricular filling pressures and stroke volume increased progressively with the load It may be concluded that on changing the heart rate selectively, regulatory mechanisms adjust the size of the stroke volume, so that cardiac output can be kept fairly constant within some limits

9 In patients with postural hypotension the aortic pressure decreased abnormally during recumbent exercise, when the cardiac output increased normally in relation to oxygen uptake The exertional hypotension, which is more pronounced in the erect position, limits the performance of muscular work The abnormal decrease in blood pressure and cardiac output, demontrated during head up tilting, indicates an impaired adjustment of both resistance and venous capacity vessels, since the application of an inflated lower body antigravity suit caused an increase of the cardiac output, but only partially restored systemic blood pressure in the head up tilted position By constant infusion of a very small dose of the sympathetic transmittor substance,

appear to affect ventricular filling at rest and during work in the recumbent position The cardiac output increased normally in relation to the oxygen uptake This was partially due to an increase of the stroke volume, which could be a consequence of the lower aortic pressure

2 The calculated total peripheral vascular resistance showed an abnormally steep decrease during recumbent exercise, due to impaired reflex control of the resistance vessels

3 The exertional hypotension, which is more marked in the erect position, is the limiting factor for performance of muscular work Probably these patients also have a low maximal heart rate

4 The abnormal decrease in systemic blood pressure and cardiac output on changing from recumbency to a head up tilted position appears to be due to an impaired adjustment both of the resistance and the capacity vessels, since the application of an inflated antigravity suit could only partially restore the blood pressure

5 A very small and well adjusted infusion of norepinephrine could essentially normalize the response to head up tilting The results are in favor of actions on resistance and capacity vessels and on myocardial contractile force

General conclusions

1 In the supine position the increase in cardiac output on transition from rest to exercise and during continued exercise up to submaximal levels is almost exclusively a result of an increase in heart rate This is true both for young nonathletes, males and females, and for athletes

2 The larger oxygen transport capacity of athletes as compared to nonathletes is chiefly explained by a larger stroke volume, which is a prerequisite for a large cardiac output during exercise and for a large mean peripheral oxygen utilization The present group of athletes had higher right ventricular and pulmonary arterial wedge pressures during work than the nonathletes

3 In the sitting position the cardiac output is less than in the supine position by about 2 l/min at rest, and during moderate and heavy exercise This difference, equal in young athletes and nonathletes, is explained by a smaller stroke volume in the sitting position, both at rest and during work

4 The stroke volume at rest is considerably less in the sitting than in the supine position It increases substantially with mild leg exercise in the sitting position After this initial change, it remains essentially constant

during continued exercise with a heavier load as in the supine position, but on a slightly lower level This is true both for athletic and nonathletic young subjects

With regard to oxygen transport, the smaller stroke volume in the sitting position is compensated for by a higher peripheral oxygen utilization, so that during work the oxygen transport per heart beat is equal in both postures In other words, the capacity for work at a given heart rate is independent of body position in normal subjects

The capacity for work at a given heart rate is dependent on body position in patients with pectus excavatum and is significantly lower in the sitting position This is explained by a deficient increase of the stroke volume on transition from rest to work in the sitting position

The stroke volume is thus abnormally small during work in the sitting position The underlying mechanism could be that in the sitting posture the right atrium rests on the depressed sternum, which then exerts a compressive effect, and/or an impaired efficiency of the abdominothoracic pumping mechanism

In the absence of valves in the leg veins, the stroke volume may fail to increase to normal extent on transition from rest to leg exercise in the erect position because of impaired ability to redistribute the gravitationally shifted blood This may reduce the capacity for work in the erect position

During constant oxygen uptake an artificially induced increase in heart rate, either by injection of an anticholinergic substance (methylscopolaminenitrate) or by raising the stimulating frequency to the ventricles in patients treated with an artificial pacemaker, caused a decrease of ventricular filling pressures and stroke volume If cardioacceleration by methylscopolaminenitrate was followed by exercise, the ventricular filling pressures and stroke volume increased progressively with the load It may be concluded that on changing the heart rate selectively, regulatory mechanisms adjust the size of the stroke volume, so that cardiac output can be kept fairly constant within some limits

In patients with postural hypotension the aortic pressure decreased abnormally during recumbent exercise, when the cardiac output increased normally in relation to oxygen uptake The exertional hypotension which is more pronounced in the erect position, limits the performance of muscular work The abnormal decrease in blood pressure and cardiac output, demontrated during head up tilting, indicates an impaired adjustment of both resistance and venous capacity vessels, since the application of an inflated lower body antigravity suit caused an increase of the cardiac output, but only partially restored systemic blood pressure in the head up tilted position By constant infusion of a very small dose of the sympathetic transmittor substance,

norepinephrine, it was possible to essentially normalize the cardiovascular response to head-up tilting. The results demonstrate the significance, for different circulatory demands, of cardiovascular control by the central reflex sympathetic mechanisms.

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